First Hit

Generate Collection | Print

L13: Entry 3 of 51 File: PGPB May 1, 2003

DOCUMENT-IDENTIFIER: US 20030083231 A1
TITLE: Blood cell deficiency treatment method

Summary of Invention Paragraph:
[0002] The invention relates to methods to make or use compounds, such as 16.alpha.-bromo-3.beta.-hydroxy-5.alpha.-androstane-17-one (16.alpha.-bromoepiandrosterone or hereafter "BrEA") 3,7,16,17-tetrahydroxyandrost-5-ene, 3,7,16,17-tetrahydroxyandrostane, 3,17-dihydroxy-16-haloandrostane, methyl 2,3,4-trihydroxy-1-O-(7,17-dioxo-androst-5-ene-3.beta.-yl)-.beta.-D-glucopyranosiduronate and related compounds. The invention relates to the use of compounds to treat a number of conditions, such as thrombocytopenia and neutropenia.

Summary of Invention Paragraph:
[0007] Hemopoiesis or hematopoiesis is the formation and development of the various types of blood cells and their progenitor cells. Mature cells are found in circulation or tissues such as the lymph nodes or the thymus. Many of the stem cells that give rise to mature forms reside in the bone marrow, although some may circulate in the blood for some time. Clinical blood cell deficiencies such as thrombocytopenia, neutropenia or erythropenia can arise from causes such as impaired hemopoiesis or abnormal loss or destruction of mature or immature blood cells.

Summary of Invention Paragraph:
[0020] Some proteins such as interleukin-6 ("IL-6"), erythropoietin ("EPO") and thrombopoietin ("TPO") have been examined for their capacity to enhance various aspects hemopoiesis, e.g., Hematology--Basic Principles and Practice, 3.sup.rd edition, R. Hoffman, E. J. Benz Jr. et al., editors, Churchill Livingstone, New York, 2000 (see, e.g., Chapter 14 at pages 154-202), O. J. Borge et al., Blood 1996 88:2859-2870, M. Cremer et al., Ann. Hematol. 1999 78:401-407, Y. Sasaki et al., Blood 1999 94:1952-1960, U.S. Pat. No. 5,879,673. Recombinant IL-6 was shown in model systems to affect platelet counts in peripheral circulation, e.g., Stahl et al., Blood 1991 78:1467-1475, although significant toxicities are associated with its administration to humans, e.g., Andus et al., FEBS Lett. 1987 221:18, J. Gauldie et al., P.N.A.S. U.S.A. 1987 84:7251-7255, T. Geiger et al., Eur. J. Immunol. 1988 18:717-721. The IL-6 molecule has been described in detail, e.g., publication no. WO 88/00206. Administration of proteins is typically expensive, given factors such as the complexity of producing pharmaceutical grade material.

Summary of Invention Paragraph:
[0022] There is a current need for cost-effective pharmaceutical agents or treatment methods that are more effective in treating deficiencies of blood cells or reducing their symptoms. The present invention provides therapeutic agents and treatment methods to treat hemopoiesis deficiencies and disorders such as TP and NP. The agents and methods are thus useful to reduce one or more symptoms associated with any of these conditions. Also, the use of the invention agents and methods can be combined with one or more conventional treatments for these disorders.

Summary of Invention Paragraph:

[0023] The invention provides a method to treat a blood cell deficiency in a subject in need thereof comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a compound of formula 1 1

Summary of Invention Paragraph:
[0119] As used herein, "innate immunity" refers to one or more components typically associated with nonspecific immune defense mechanisms in a subject. These components include the alternate complement pathway, e.g., Factor B, Factor D and properdin; NK cells, phagocytes (monocytes, macrophages), neutrophils, eosinophils, dendritic cells, fibrocytes; anti-microbial chemicals, e.g., one or more of defensins; physical barriers--skin, mucosal epithelium; or certain interleukins, chemokines, cytokines, lung or alveolar macrophage respiratory burst activity or a lung surfactant protein such as surfactant protein A or surfactant protein D. Innate immunity plays a role in resistance to intracellular parasite infections, e.g., white blood cell infection, a liver infection, and other infections, e.g., lymph node infections. Detectable enhancement of innate immunity mechanism by formula 1 compounds or method described herein can also enhance phagolysosome fusion or movement, which some pathogens, e.g., intracellular bacteria such as mycobacteria, or Listeria inhibit.

Summary of Invention Paragraph:
[0127] Terms such as "biologically active metabolite" and the like mean derivatives of the formula 1 compounds that retain a detectable level, e.g., at least about 10%, at least about 20%, at least about 30% or at least about 50%, of at least one desired activity of the parent compound, e.g., antiinflammatory activity or stimulation of a desired immune response. Determination of a desired activity is accomplished essentially as described herein. Such metabolites can be generated in the gastrointestinal tract, in blood or in one or more subject tissues. Such metabolites are detected using standard analytical methods, e.g., GC-MS analysis of an optionally radiolabeled formula 1 compound and its metabolites, in blood, urine or other biological samples after it is administered to a subject by one or more routes as disclosed herein. Terms such as "metabolic precursor" of formula 1 compounds and the like can include compounds that generate a detectable level of the formula 1 compound or a detectable level, e.g., at least about 10%, at least about 20%, at least about 30% or at least about 50%, of at least one desired activity of the formula 1 compound. Determination of a desired activity is accomplished essentially as described herein. Conversion of metabolic precursors can occur in the gastrointestinal tract, in blood or in one or more subject tissues.

Summary of Invention Paragraph:
[0191] Hydrolyzable moieties typically comprise acyl groups, esters, ethers, thioethers, amides, amino acids, peptides, carbonates and/or carbamates. In general, the structure of hydrolyzable moieties is not critical and can vary. In some embodiments, these moieties contain a total of about 4 to about 10 carbon atoms. These hydrolyzable moieties in other embodiments comprise an organic moiety, as described above for ester, that contains 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 13, 14, 15 or 16 carbon atoms and 1, 2, 3, 4, 5, 6, 7 or 8 heteroatoms, e.g., oxygen, nitrogen or sulfur. These hydrolyzable moieties can comprise no groups that are charged in plasma, blood, intracellular cytoplasm or in the gut, or they can comprise 1, 2, 3 or more positive, negative or positive and negative charges under one or more of these conditions. The charges may be fractional depending on the group and the conditions it is under. These hydrolyzable moieties may comprise 1, 2, 3, 4 or more substitutions at a hydrogen atom(s) and/or a carbon atom(s), e.g., --OH, protected hydroxyl, --SH, protected thiol, carboxyl, protected carboxyl, amine, protected amine, --O--, --S--, --CO--, --CS--, alkoxy, alkylthio, alkenyloxy, aryl, --OP(O)(O)--O--, --OS(O)(O)--O-- and/or heterocycle. Such substitutions are independently selected. Embodiments of formula 1 compounds include ones wherein one, two, three, four or more of the variable groups that are bonded to the steroid rings, e.g., R.sup.1--R.sup.6 or R.sup.10, comprise a moiety

that can hydrolyze or metabolize to, e.g., a --H, --OH, .dbd.O, --SH, .dbd.S, --COOH, --NH.sub.2, --CH.sub.2OH, --CH.sub.2SH, --C(O)--C.sub.1-6 alkyl-OH, --C(O)--C.sub.1-6 alkyl-SH, --C(S)--C1-C6 alkyl-OH, --C(O)--C1-C6 alkyl or --C(O)--NH.sub.2 atom or group.

Summary of Invention Paragraph:
[0522] The individual compounds and genera named in groups 1-54 above may also be named using any suitable formal or informal chemical nomenclature. Thus, as will be apparent, individual compounds in these groups incude 16.alpha.-bromoepiandrosterone, 16.alpha.-hydroxyepiandrost- erone, 3.alpha.,16.alpha.-dihydroxy-5.alpha.-androstane-17-one, 3.alpha.,16.alpha.,17.alpha.-trihydroxy-5.alpha.-androstane, 3.alpha.,16.alpha.,17.alpha.-trihydroxy-5.alpha.-androstane, 3.beta.,17.beta.-dihydroxyandrost-5-ene or 3.beta.,7.beta.,17.beta.-trihydroxyandrost-5-ene, 7-oxodehydroepiandrosterone, 16.alpha.-fluoroandrost-5- -ene-17-one, 7.alpha.-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 7.beta.-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 17.alpha.-hydroxy-16.alpha.-fluoroandrost-5-ene, 17.beta.-hydroxy-16.alph- a.-fluoroandrost-5-ene and the like.

Summary of Invention Paragraph:
[0544] wherein (i) R.sub.7 is an alkyl ester wherein the alkyl is optionally substituted, and (ii) R.sub.8, R.sub.9 and R.sub.10 are each --OR.sub.14, wherein R.sub.14 is a hydrogen atom or a protected hydroxy; and (iii) at least one of R.sub.1 or R.sub.2 is not hydrogen; (b) R.sub.5 and R.sub.6 are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy; or R.sub.5 and R.sub.6 taken together form an oxygen atom, which, together with the carbon atom to which R.sub.5 and R.sub.6 are joined, forms a ketone group; and (c) R.sub.12 and R.sub.13 are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl (e.g., methyl, ethyl or --CHO), hydroxy, and a protected hydroxy. Such compounds include ones wherein (1) the protected hydroxy is an ester such as an acetate or proprionate, (2) one of R.sub.1 and R.sub.2 is a hydrogen atom and the other one of R.sub.1 and R.sub.2 is said glucuronide, (3) R.sup.5 and R.sub.6 are each independently selected from the group consisting of a hydrogen atom, hydroxy, and acetate, e.g., one of R.sub.5 and R.sub.6 is a hydrogen atom and the other is acetate, (4) R.sub.12 and R.sub.13 are methyl, and/or (5) R.sub.7 is a methyl ester. These compounds include ones where R.sub.1 is a glucuronide group, R.sub.2 is a hydrogen atom, and R.sub.5 and R.sub.6 together are .dbd.O and methyl 2,3,4-trihydroxy-1-O-(7,17-dioxoan- drost-5-ene-3.beta.-yl)-.beta.-D-glucopyranosiduronate and methyl 2,3,4-tri-O-acetyl-1-O-(7,17-dioxoandrost-5-ene-3.beta.-yl)-,-D-glucopyra- nosid-uronate, or a pharmaceutically acceptable salt, ester, ether, amide, or prodrug thereof.

Summary of Invention Paragraph:
[0548] wherein (i) R.sub.7 is an alkyl ester wherein the alkyl is optionally substituted, and (ii) R.sub.8, R.sub.9 and R.sub.10 are each --OR.sub.14, wherein R.sub.14 is a hydrogen atom or a protected hydroxy; and (iii) at least one of R.sub.5 and R.sub.6 is not hydrogen; (b) R.sub.11 is a hydrogen atom or a protected hydroxy; and (c) R.sub.12 and R.sub.13 are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy. Such compounds include ones wherein (1) the protected hydroxy may be an ester, e.g., acetate or proprionate, (2) one of R.sub.5 and R.sub.6 is a hydrogen atom and the other one of R.sub.5 and R.sub.6 is a glucuronide, (3) R.sub.5 is a glucuronide group and R.sub.6 is a hydrogen atom, (4) R.sub.12 and R.sub.13 are methyl, and/or (5) R.sub.7 is methyl ester. These compounds include methyl-2,3,4-trihydroxy-1--O---(3.beta.-.a- lpha.cetoxyandrost-5-ene-7-oxo-17.beta.-yl)-.beta.-D-glucopyranosiduronate and methyl-2,3,4-tri-O-acetyl-1-O-(3.beta.-.alpha.cetoxyandrost-5-ene-7-o- xo-17.beta.-yl)-.beta.-D-glucopyranosiduronate, or a pharmaceutically acceptable salt, ester, ether, amide, or prodrug thereof.

Summary of Invention Paragraph:
[0552] wherein (i) R.sub.7 is an alkyl ester wherein the alkyl is optionally substituted, and (ii) R.sub.8, R.sub.9 and R.sub.10 are each --OR.sub.14, wherein R.sub.14 is a hydrogen atom, optionally substituted alkyl, cycloalkyl, e.g., C3, 4, 5, 6, 7, or 8 cycloalkyl, or a protected hydroxy; and (iii) at least one of R.sub.3 and R.sub.4 is not hydrogen; (b) R.sub.5 and R.sub.6 are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy; or R.sub.5 and R.sub.6 taken together form an oxygen atom, which, together with the carbon atom to which R.sub.5 and R.sub.6 are joined, forms a ketone group; (c) R.sub.11 is a hydrogen atom or a protected hydroxy; and (d) R.sub.12 and R.sub.13 are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy. Such compounds include ones wherein (1) the protected hydroxy may be an ester, e.g., acetate or proprionate, (2) R.sub.3 and R.sub.4 are --H and a glucuronide, (3) R.sub.5 and R.sub.6 are each independently selected from the group consisting of --H, hydroxy, and acetoxy, e.g., they are --H and acetoxy, (4) R.sub.12 and R.sub.13 are methyl and/or R.sub.7 is methyl ester. Such compounds include ones wherein R.sub.3 is a glucuronide group, R.sup.4 is --H, R.sub.5 is acetate, or wherein R.sub.6 is --H and wherein R.sub.3 is --H, R.sub.4 is a glucuronide group, and R.sub.5 and R.sub.6 together form .dbd.O, and methyl-2,3,4-tri-O-acetyl-1-O-(3.beta.,- 17.beta.-diacetoxyandrost-5-ene-7.beta.-yl)-.beta.-D-glucopyranosiduronate- , methyl 1-O-(3.beta.,17.beta.-diacetoxyandrost-5-ene-7.beta.-yl)-.beta.-D- -glucopyranosiduronate, and methyl-2,3,4-tri-O-acetyl-1-O-(3.beta.-acetoxy- -17-oxoandrost-5-ene-7.alpha.-yl)-.beta.-D-glucopyranosiduronate, or a pharmaceutically acceptable salt, ester, ether, amide, or prodrug thereof.

Summary of Invention Paragraph:
[0555] wherein (a) R.sub.1 and R.sub.2 are each independently selected from the group consisting of a hydrogen atom and --OR.sub.14, wherein (i) R.sub.14 is selected from the group consisting of a hydrogen atom, optionally substituted alkyl, and a protected hydroxy; and (ii) at least one of R.sub.1 or R.sub.2 is not hydrogen; (b) R.sub.5, R.sub.6, R.sub.7, and R.sub.8 are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, and a protected hydroxy; or R.sub.5 and R.sub.6 taken together form an oxygen atom, which, together with the carbon atom to which R.sub.5 and R.sub.6 are joined, forms a ketone group; or R.sub.7 and R.sub.8 taken together form an oxygen atom, which, together with the carbon atom to which R.sub.7 and R.sub.8 are joined, forms a ketone group; and (c) R.sub.12 and R.sub.13 are each independently selected from the group consisting of a hydrogen atom, alkyl, hydroxy, and a protected hydroxy. Such compounds include ones wherein (1) the protected hydroxy is an ester, e.g., acetate or proprionate, or a trialkylsilyl, e.g., t-butyldimethylsilyl, and wherein R.sub.1 and R.sub.2 are --H and --OR.sub.14 where R14 is optionally selected from methyl, ethyl, n-propyl, i-propyl, n-butyl, sec-butyl, t-butyl, pentyl, hexyl, n-octyl, n-dodecyl, 1-ethoxyethyl, t-butyldimethylsilyl, tetrahydropyran-2-yl, and --C(O)CH.sub.3, (2) R.sub.5 and R.sub.6 are each independently selected from the group consisting of --H, --OH, and trialkylsilyl, e.g., R.sub.5 and R.sub.6 are --H and a trialkylsilyl or R.sub.5 and R.sub.6 are --H and --OH or R.sup.5 and R.sub.6 together are .dbd.O, (3) R.sub.12 and R.sub.13 are methyl, and/or (4) R.sub.7 and R.sub.8 are independently --H, --OH or trialkylsilyl, e.g., R.sub.7 and R.sub.8 are --H and a trialkylsilyl or R.sub.7 and R.sub.8 are --H and --OH or R.sub.7 and R.sup.8 together are .dbd.O. These compounds include 3.beta.-tosyloxyandrost-5-ene-17-one, 3.beta.-methoxyandrost-5-ene-17-one, 3.beta.-methoxyandrost-5-ene-7,17-di- one, 3.beta.-methoxy-17,17-ethylenedioxyandrost-5-ene-7-one, 3.beta.-methoxy-17,17-ethylenedioxyandrost-5-ene-7.beta.-ol, 3.beta.-methoxy-17,17-ethylenedioxyandrost-5-ene-7.alpha.-ol, 3.beta.-methoxyandrost-5-ene-7.beta.,17.beta.-diol, 3.beta.-acetoxy-7.alpha.-bromoandrost-5-ene-17-one, 3.beta.-acetoxy-7-methoxyandrost-5-ene-17-one, 3.beta.-methoxyandrost-5-e- ne-17.beta.-ol, 3.beta.-methoxy-17.beta.-hydroxyandrost-5-ene-7-one, 3.beta.-methoxy-17.beta.-acetoxyandrost-5-ene-7-one, 3.beta.-t-butoxyandrost-5-ene-17-one, 3.beta.-t-butoxyandrost-5-ene-7,17-- dione, 3.beta.-t-

butyldimethylsilyloxyandrost-5-ene-7,17-dione, 3.beta.,17.beta.-di(t-butyldimethylsilyloxy)androst-5-ene-7-one, 3.beta.-acetoxyandrost-5-ene-7.beta.,17.beta.-diol, 3.beta.-acetoxyandrost-5-ene-7.beta.,17.beta.-di(t-butyldimethylsilyl) ether, 3.beta.-acetoxy-17.beta.-t-butyldimethylsilyloxyandrost-5-ene-7-on- e, 3.beta.-(2-tetrahydropyranoxy)androst-5-ene-7,17-dione, 3.beta.-dodecanoxyandrost-5-ene-17-one, 3.beta.-dodecanoxyandrost-5-ene-7- ,17-dione, 3.beta.-(1'-ethoxy)ethoxyandrost-5-ene-17-one, and 3.beta.-(1'-ethoxy) ethoxyandrost-5-ene-7,17-dione, or the pharmaceutically acceptable salt, ester, ether, amide, or prodrug thereof.

Summary of Invention Paragraph:
[0558] wherein (a) R.sub.1 and R.sub.2 are each independently selected from the group consisting of a hydrogen atom and --O--C(O)--OR.sub.14, wherein (i) R.sub.14 is selected from the group consisting of a hydrogen atom, optionally substituted alkyl, and a carbocyclic ring; and (ii) at least one of R.sub.1 or R.sub.2 is not hydrogen; (b) R.sub.5, R.sub.6, R.sub.7, and R.sub.8 are each independently selected from the group consisting of a hydrogen atom, optionally substituted alkyl, hydroxy, --O--C(O)--OR.sub.14, and a protected hydroxy; or R.sub.5 and R.sub.6 taken together form an oxygen atom, which, together with the carbon atom to which R.sub.5 and R.sub.6 are joined is .dbd.O (a ketone); or R.sub.7 and R.sub.8 taken together form an oxygen atom, which, together with the carbon atom to which R.sub.7 and R.sub.8 are joined is .dbd.O; and (c) R.sub.12 and R.sub.13 are each independently selected from the group consisting of a hydrogen atom, alkyl, hydroxy, and a protected hydroxy. Such compounds incl;ude ones wherein (1) the protected hydroxy is an ester, e.g., acetate or proprionate, (2) R.sub.1 and R.sub.2 are --H and --O--C(O)--OR.sub.14 and R.sub.14 optionally is methyl, ethyl, propyl, n-butyl, sec-butyl, t-butyl, n-octyl, n-dodecyl, 1-ethoxyethyl, 9-fluorenylmethyl or --C(O)CH.sub.3, (3) R.sub.5 and R.sub.6 independently are --H, --OH, --O--C(O)--OCH.sub.3, --O--C(O)--OC.sub.2H.sub.5, --O--C(O)--OC.sub.3H.sub.7, --O--C(O)--OC.sub.4H.sub.9, --O--C(O)--OCH.sub.2C.sub.2H.sub.3, --O--C(O)--OCH.sub.2C.sub.3H.sub.5 or --O--C(O)--O--(CH.sub.2).sub.2--O--- C.sub.2H.sub.5, (3) R.sub.5 and R.sub.6 are --H and --OH, e.g., R.sub.5 and R.sub.6 are --H and --OH or together are .dbd.O, (4) R.sub.12 and R.sub.13 are methyl, (4) R.sub.7 and R.sub.8 are each independently selected from the group consisting of a hydrogen atom, hydroxy, and trialkylsilyl, e.g., R.sub.7 and R.sub.8 are are --H and --OH or together are .dbd.O. These compounds include 3.beta.-carbomethoxyandrost-5-ene-7,17-dione, 3.beta.-carboallyloxyandrost-5-ene-7,17-dione, 3.beta.-carboethoxyandrost-5-ene-7,17-dione, 3.beta.-carboisobutoxyandros- t-5-ene-7,17-dione, 3.beta.,17.beta.-dicaromethoxyandrost-5-ene-7-one, 3.beta.-carbooctyloxyandrost-5-ene-7,17-dione, 3.beta.-carbo(9-fluorenyl)- methoxyandrost-5-ene-7,17-dione, 3.beta.-carbomethoxyandrost-5-ene-7,17.be- ta.-diol, 3.beta.-carboethoxyandrost-5-ene-7.beta.,17.beta.-diol, and 3.beta.-carbooctyloxyandrost-5-ene-7.beta.,17.beta.-diol, or an pharmaceutically acceptable salt, ester, ether, amide, or prodrug thereof.

Summary of Invention Paragraph:
[0567] One aspect of invention intermittent dosing is monitoring the subject's response to a particular dosing regimen or schedule, e.g., to any intermittent administration method disclosed herein. For example, while dosing a subject who has a viral infection (e.g., HCV, HIV, SIV, SHIV), one can measure the subject's or pathogen's response, e.g., amelioration of one or more symptoms or a change in infectious particles or viral DNA or RNA in the serum or a change in an immune parameter of interest. Once a response is observed dosing can be continued for one, two or three additional days, followed by discontinuing the dosing for at least one day (at least 24 hours), usually for at least about 2, 3, 4, 5, 6, 7, 14, 21, 28, 42, 56, 70, 84, 98, 112 or more days. Once the subject's response shows signs of remission (e.g., a symptom begins to intensify, viral serum DNA or RNA begins to increase or an immune parameter, e.g., as described herein, begins to deteriorate), dosing can be resumed for another course. An aspect of the subject's response to formula 1 compound(s) is that the subject may show a measurable response within a

short time, usually about 5-10 days, which allows straightforward tracking of the subject's response, e.g., by monitoring viral titer in peripheral white blood cells ("PBMC"), by measuring viral nucleic acid levels in the blood or by measuring a white blood cell population(s) or expression of a cytokine or interleukin by e.g., white blood cells or a subset(s) thereof. One may monitor one or more immune cell subsets, e.g., NK, LAK, dendritic cells or cells that mediate ADCC immune responses, during and after intermittent dosing to monitor the % 20 subject's response and to determine when further administration of the formula 1 compound is indicated. These cell subsets are monitored as described herein, e.g., by flow cytometry.

Summary of Invention Paragraph:
[0641] Also falling within the scope of this invention are the in vivo metabolites of the compounds described herein and the use of the metabolites for use in the therapeutic treatments or other methods described herein or in the cited references. This includes metabolites or products that are novel and unobvious over the prior art as new compounds as such and their uses. Metabolites may result for example from the oxidation, reduction, hydrolysis, amidation, esterification, glycosidation and the like of the administered formula 1 compound, due to enzymatic or chemical processes. Metabolites may be generated in vivo in a subject or they may arise ex vivo from cells or tissues, e.g., from a mammal such as a human, rodent or a primate. Accordingly, the invention includes novel and unobvious compounds produced by a process comprising contacting a compound of this invention with a subject or a subject's cells or tissue for a period of time sufficient to yield detectable amounts of a metabolic product thereof. Such products typically are identified by preparing a radiolabeled or mass labeled formula 1 compound that comprises, e.g., 1, 2, 3 or more .sup.13C, .sup.14C, .sup.3H, .sup.2H, .sup.113I, .sup.32P, .sup.35S or .sup.99Tc atoms bonded to the compound, and administering it as a trace labeled compound along with the unlabeled compound. The labeled and unlabeled compound is administered by any suitable route (by, e.g., a buccal, sublingual, parenteral, topical or oral route) in a detectable dose (e.g. greater than about 0.1 .mu.g/kg, or at least about 10 .mu.g/kg or at least about 0.5 mg/kg of the labeled compound) to a subject, e.g., an animal or mammal such as rat, mouse, guinea pig, primate, or to a human. After administration sufficient time is allowed for metabolism to occur (typically about 30 seconds to 30 hours) and conversion products are isolated from one or more of the urine, blood, plasma, feces or other suitable biological sources. The amount of labeled formula 1 compound that is administered to a subject will vary with the specific activity of the labeled compound. Exemplary metabolic conversions of formula 1 compounds include modification of hydrogen atoms or other moieties that are bonded to, e.g., one or more of the 1, 2, 3, 4, 6, 7, 11, 15, 16 or 17 positions. Exemplary conversions at these one or more of positions include hydroxylation of ring atoms, e.g., ring carbon atoms, conjugation of hydroxyl groups that are bonded to one or more of those positions with moieties such as sulfate, phosphate or a monosaccharide or disaccharide such as glucuronic acid and hydrolysis of moieties such as esters or alkoxy groups.

Summary of Invention Paragraph:
[0660] Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats, salts (e.g., NaCl, potassium or sodium carbonate or bicarbonate or potassium or sodium phosphates) and solutes which render the formulation isotonic with the blood of the intended subject; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. In general, the formula 1 compound that is present in liquid compositions or formulations is completely dissolved in aqueous or non-aqueous excipients. However, in some embodiments, e.g., transient compositions or some formulations, the formula 1 compound is partially dissolved while the remaining portion is present as a solid, which can be a suspension or a colloid.

Summary of Invention Paragraph:
[0706] As used herein, reference to Th1 or Th2 immune responses means such responses as observed in mammals generally and not as observed in the murine system, from which the Th1 and Th2 terminology originated. Thus, in humans, Th1 cells are CD4.sup.+ T lymphocytes and they usually preferentially display chemokine receptors CXCR3 and CCR5, while Th2 cells are CD4.sup.+ T lymphocytes and usually preferentially express the CCR4, CCR8 and/or CXCR4 chemokine receptor molecule(s) and generally a smaller amount of CCR3, see, e.g., U. Syrbe et al., Springer Semin. Immunopathol. 1999 21:263-285, S. Sebastiani et al., J. Immunol. 2001166:996-1002. Tc1 and Tc2 immune responses are mediated by CD8.sup.+ lymphocytes and means to identify these cells and their associated lymphokines, cell specific antigens and biological activities have been described, see, e.g., M. B. Faries et al., Blood 200198:2489-2497, W. L. Chan et al., J. Immunol. 2001167:1238-1244, C. Prezzi et al., Eur. J. Immunol. 200131:894-906, H. Ochi et al., J. Neuroimmunol. 2001119:297-305, D. H. Fowler and R. E. Gress, Leukemia and Lymphoma 2000 38:221-234.

Summary of Invention Paragraph:
[0709] The invention provides a method to detectably enhance an antigen specific immune response, cell mediated immune response or a delayed-type hypersensitivity immune response in a subject having impaired or negligible antigen specific immune response, cell mediated immune response or delayed-type hypersensitivity immune response, comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a formula 1 compound. The antigen specific immune response, cell-mediated immune response or delayed-type hypersensitivity immune response can be enhanced at least about 25%, at least about 40%, at least about 50%, at least about 60%, at least about 75% or at least about 90%. Some of the subjects may have an antigen specific immune response, cell mediated immune response or a delayed-type hypersensitivity immune response that is impaired or negligible, e.g., about 50% or less or about 30% or less or about 10% or less of the response that an otherwise normal subject would be expected to have. Such subjects may not detectably respond to at least 1 antigen out of 2, 3, 4 or 5 antigens that a normal subject would respond to. In some embodiments, the subject is an HIV-infected human having a CD4.sup.+ T cell count of about 0-150 cells/mm.sup.3 or about 2-100 cells/mm.sup.3 and/or wherein the antigen specific immune response, cell mediated immune response or delayed-type hypersensitivity immune response is an enhanced response to a viral, bacterial, parasite or fungal antigen such as an HIV, HCV, HBV or CMV antigen such as a viral or HIV core antigen or HIV p24 antigen or a viral or HIV envelope antigen, a Candida antigen, a viral, bacterial, parasite or fungal antigen essentially as described herein or to phytohemagglutinin. The responses to treatment with a formula 1 compound may be quantitated by, e.g., mixed lymphocyte reaction, ELIspot analysis or flow cytometric analysis of, e.g., circulating blood cells such as CD4+ or CD8.sup.+ T cells or for levels of cytokines (e.g., IL-2, TNF.alpha. or IFN.gamma.) in such cells. Such analyses have been described, e.g., V. P. Badovinac and J. T. Hardy, J. Immunol Methods 2000, 238:107-117, N. Favre et al., J. Immunol. Methods 1997, 204:57-66, E. Hagiwara et al., Cytokine 1995, 7:815-822, N. W. Lukacs et al., Blood 1993, 82:3668-3674, M. Umemoto et al., Clin. Exp. Immunol. 1998, 112:459-463, A. Fietta et al., Gerontology 1994, 40:237-245, C. H. Orteu et al., J. Immunol. 1998, 161:1619-1629.

Summary of Invention Paragraph:
[0713] Aspects of the invention include the use or administration of compositions or formulations that comprise a carrier and an amount of at least one formula 1 compound effective to detectably modulate an immune parameter. For example, to enhance the relative proportion of a desired immune cell subset, e.g., CD4.sup.+ T cells, CD8.sup.+ T cells, NK cells, LAK cells, neutrophils, granulocytes, basophils, eosinophils or dendritic cells, or to modulate (detectably increase or decrease) one or more functions of immune cell subsets. The formula 1 compounds can modulate the expression of CD molecules or alter the proportion of cell subsets, e.g., CD4+ or CD8.sup.+ T cells, or their relative numbers in a subject's blood or

tissues. CD and related molecules participate in the function of various immune cell subsets and can be useful as markers for immune function in vivo. In some aspects, the formula 1 compounds activate immune cells which generally alters (increases or decreases) expression of, or changes the numbers of cells that express one or more of, CD4, CD6, CD8, CD25, CD27, CD28, CD30, CD36, CD38, CD39, CD43, CD45RA, CD45RO, CD62L, CD69, CD71, CD90 or HLA-DR molecules. Often, the numbers of cells that express these molecules are increased, e.g., CD25, CD16 or CD69. Typically, such increases are observed as an increased proportion of circulating white blood cells that express one or more of these molecules or white blood cells CXCR3, CCR5, CCR4, CCR8 and/or CXCR4. In some cases the number of such molecules per cell is detectably altered.

Summary of Invention Paragraph:
[0714] Expression of one or more adhesion molecules CD2, CD5, CD8, CD11a, CD11b, CD11c, CD18, CD29, CD31, CD36, CD44, CD49a, CD49b, CD49c, CD49d, CD49e, CD49f, CD50, CD54, CD58, CD103 or CD104 are also detectably modulated after administration of the formula 1 compounds to a subject. Often, the numbers of cells that express these molecules are increased, e.g., CD5 or CD56. The adhesion molecules function in various aspects of immune responses, such as binding to class I MHC molecules, transducing signals between cells or binding to molecules in the extracellular matrix associated with endothelial or other cell types. Administration of the formula 1 compounds to a subject also affects the numbers of certain immune cell subsets, e.g., NK cells (e.g., CD8.sup.=, CD56.sup.+ or CD8.sup.+, CD56.sup.+) or lymphokine activated killer cells (LAK). Increased circulating NK or LAK cells are typically observed, which is reflected in increased numbers of cells that express one or more of CD16, CD38, CD56, CD57 or CD94. Also, increased numbers of circulating dendritic cell precursors are observed, as shown by increases in cells that express one or more of CD11c, CD80, CD83, CD106 or CD123. Although one can observe an increased proportion of circulating white blood cells that express one or more of these molecules, in some instances the number of such molecules per cell is detectably altered. Both the cell numbers and the density of CD molecule per cell can also be detectably modulated. Modulation of immune cell subsets typically occurs on intermittent dosing of a formula 1 compound, but will arise from any suitable dosing regimen, e.g., as described herein.

Summary of Invention Paragraph:
[0716] Thus, in some embodiments, the migration of one or more immune cell subsets such as CD11C.sup.+ cells from tissue such as skin or lung through the blood to immune tissue such as lymph nodes or GALT is seen as a transient increase in the level of circulating CD11C.sup.+ cells in response to exposure of the subject's tissues to a suitable amount of a formula 1 compound. Thus, the level of CD11C.sup.+ cells in the blood will generally detectably increase, e.g., a statistically significant increase, plateau and then decrease as migration of the cells to immune tissue subsides. In these embodiments, the proportion of the cells of the affected immune cell subset is typically relatively low in most physiological immune states, e.g., normal or abnormal immune conditions, compared to the total white blood cell population in circulation. In other embodiments, the migration of one or more immune cell subsets such as CD123.sup.+ cells from the circulation to immune tissue such as lymph nodes or GALT results in a decrease. In these embodiments, the decrease in the numbers of circulating immune cells reflects the migration of the immune cells from the blood to immune tissue such as lymph nodes or GALT. Such a decrease may be transient and followed by recovery of the affected immune cell subset(s) over about 2 to 24 weeks. In conducting these embodiments, administration of the formula 1 compound to the subject is accomplished using the formulations or the methods as described herein.

Summary of Invention Paragraph:
[0717] Thus, an aspect of the invention is a method to enhance the migration of one or more immune cell types in a subject from one location (e.g., bone marrow, circulating blood or a tissue such as the skin, liver, central nervous system or

lung) to another (e.g., to the blood or to a lymphoid tissue such as a lymph node, spleen or a mucosal tissue such as GALT) by administration to a subject as described herein of an effective amount of a formula 1 compound essentially as described by any of the methods disclosed herein. A related aspect is the monitoring, e.g., by suitable blood counts or tissue biopsy, of the subject's response to determine the timing and extent of such immune cell migration.

Summary of Invention Paragraph:
[0719] Treatment of a subject with a formula 1 compound can result in a change of at least about 20-80% or about 25-50% above or below (e.g., at least 30% or at least 40% above or below) the control or basal level of affected immune cell subsets. For example, increases of more than about 30% in the total numbers of activated CD8.sup.+ T cells, e.g., CD8.sup.+, CD69.sup.+, CD25.sup.+ T cells, CD8.sup.+, CD69.sup.+, CD25.sup.- T cells or CD8.sup.+, CD69.sup.-, CD25.sup.+ T cells, can occur by 7 days after a single dose of a formula 1 compound to a subject. Such increases may be greater than 50%, 60% or 100% in the total numbers of activated CD8.sup.+ T cells or subsets of activated CD8.sup.+ T cells in individual subjects. Typically such increases are about in the total numbers of activated CD8.sup.+ T cells or subsets of activated CD8.sup.+ T cells averages about 30-40%, with individual subjects experiencing increases over 100% in the numbers of activated CD8.sup.+ T cells per unit blood volume compared to the basal level.

Summary of Invention Paragraph:
[0729] In an exemplary embodiment, human patients infected with HCV are dosed with an aqueous isotonic .alpha.-cyclodextrin or .beta.-cyclodextrin, e.g., hydroxypropyl-.beta.-cyclodextrin, formulation containing about 20 mg/mL BrEA. The formulation is delivered intravenously in a single daily dose or two subdoses per day. The patients are dosed with 1 to 10 mg/kg/day for 4 to 10 days, followed by no dosing for 5 to 30 days, followed by dosing again with the cyclodextrin formulation for 4 to 10 days. The dosing regimen is repeated one, two or more times. Clinical markers for HCV infection are followed during treatment, e.g., viral nucleic acid in the blood or plasma, liver enzyme levels in the blood or plasma (e.g., AST/SGOT, ALT/SGPT, alkaline phosphatase). For these patients, an anti-HCV treatment(s), e.g., .gamma.IFN, .alpha.IFN, a retroviral protease inhibitor, a nucleoside analog, and/or ribavirin, is optionally started or continued according to the recommendations of the patient's doctor and with the patient's informed approval. In some of these embodiments, a formula 1 compound(s) is administered daily continuously as a component in an oral or parenteral composition or formulation, e.g., for a formula 1 compound(s) that is a new compound per se. BrEA is optionally also administered systemically using, e.g., a parenteral formulation to deliver 0.1-5 mg/kg/day either daily or every other day for about 1 to 4 months, or an oral formulation to deliver about 0.5-40 mg/kg/day either daily or every other day for about 1 to 4 months.

Summary of Invention Paragraph:
[0732] Hantavirus infection is a viral disease that rodents can transmit to humans and the infection is associated with serious lung or kidney infection. Symptoms of Hantavirus infection of the lungs include one or more of fever, muscle pain, myalgia, headache, abdominal pain, conjunctival bleeding, diarrhea, vomiting, coughing, shortness of breath or low blood pressure (shock). Hantavirus kidney infection may be mild or severe and is associated with fever, headache, backache, abdominal pain, small bruise-like patches on the whites of the eyes, abdominal rash, impaired kidney function, nausea, loss of appetite, fatigue and intracranial bleeding.

Summary of Invention Paragraph:
[0743] Similarly, the formula 1 compounds can be used to treat, prevent or ameliorate an infection by one or more gram-negative enteric bacteria. Such bacteria are commonly members of the Bartonella, Brucella, Campylobacter,

Enterobacter, Escherichia, Francisella, Klebsiella, Morganella, Proteus, Providencia, Pseudomonas, Salmonella, Serratia, Vibrio or Yersinia genera. For these infections, the formula 1 compound is administered to a subject such as a human at a dosage disclosed herein, e.g., about 0.5-4 mg/kg/day by buccal delivery or by a parenteral route such as subcutaneous, intramuscular or intravenous injection, for about 5-14 consecutive days. An oral dosage would be about 10-25 mg/kg/day of a formula 1 compound for about 5-14 consecutive days. Typically dosing with the formula 1 compound will begin at the time that (or shortly thereafter, e.g., within about 1-12 hours) the infection is suspected or is diagnosed. The patient is optionally monitored and the amelioration of one or more symptoms or a slowed disease progression is observed. The compounds can reduce the adverse effects of bacterial lipopolysaccharide or endotoxin that is associated with these organisms. For example, the compounds are therapeutically useful for infection by Yersinia pestis, which causes plague. Several forms of plague can exist, i.e., bubonic, pneumonic, septicemic, or pestis minor. The compounds ameliorate one or more of the symptoms associated with these infections. For example, in a bubonic plague infection, symptoms typically arise several days after exposure to Y. pestis, and can include a fever of up to 106.degree. F., chills, rapid weak heartbeat, low blood pressure, lymph node swelling accompanied by tenderness, restlessness, confusion, uncoordinated movements, liver and spleen swelling. Symptoms associated with pneumonic plague include high fever, chills, rapid heartbeat, severe headache, coughing, blood-tinged sputum and rapid and labored breathing.

Summary of Invention Paragraph:
[0753] When treating other viral infections of the respiratory system, liver, blood, skin or other systems, e.g., human hepatitis C virus ("HCV"), human hepatitis B virus ("HBV"), HIV-1, HIV-2, an Orthopoxyirus infection, a filovirus infection, a picornavirus infection or an influenza virus infection (e.g., human influenza A or B), a formula 1 compound are optionally is used in conjunction with antivirals or treatments for such viruses. Examples of such therapeutic agents or treatments which are useful in these methods include carbovir, oxathiolan nucleoside analogs such as cis-1-(2-hydroxymethyl)-1,3-oxathiolan-5-yl)-c- ytosine or cis-1-(2-hydroxymethyl)-1,3-oxathiolan-5-yl-5-fluoro-cytosine, 2',3'-didoxy-5-ethynyl-3'-fluorouridine, 5-chloro-2',3'-didoxy-3'-fluorou- ridine, 1-(.beta.-D-arabinofuranosy)-5-propynyluracil, tenofovir, tenofovir disoproxil, tenofovir disoproxil fumarate, bis(POC)-PMEA, bis(POM)-PMEA, bis(POC)-PMPA, bis(POM)-PMPA, acyclovir, HPMPC, amantadine, rimantadine, ribavirin, oseltamivir or compounds disclosed in U.S. Pat. Nos. 5,763,483 (especially compounds recited in claims 1 and 2), 5866601 and 6043230, mucolytics, expectorants, bronchodilators, antibiotics, antipyretics, analgesics or cytokines or interleukins that can augment one or more aspects of a desired immune response, e.g., IL-1, IL-2, IL-3, IL-6, .alpha.-interferon, .beta.-interferon, .gamma.-interferon, G-CSF, GM-CSF, M-CSF or thrombopoietin. Such cytokines or interleukins can be used for any viral infection essentially according to known dosing methods and dosages, e.g., as disclosed herein or in the cited references.

Summary of Invention Paragraph:
[0758] Examples of such agents or treatments include the use of one or more adrenergic agents, adrenocortical suppressants, aldosterone antagonists, anabolics, analeptics, analgesics, anesthesia, anthelmintics, antiacne agents, anti-adrenergics, anti-allergics, anti-amebics, anti-androgens, antianginals, anti-anxiety agents, anti-arthritics, anti-asthmatic agents, anti-atherosclerotic agents, antibacterials, anticholinergics, anticoagulants, anticonvulsants, antidepressants, antidiabetics, antidiarrheals, antidiuretics, anti-emetics, anti-epileptics, anti-estrogens, antifibrinolytics, antifungals, antihistamines, antihyperlipidemia agents, antihyperlipoproteinemic agents, antihypertensive agents, antihypotensives, anti-infectives, anti-inflammatory agents such as entanercept (a dimeric fusion coprising a portion of the human TNF receptor linked to the Fc protion of human IgG1 containing the C.sub.H2 and C.sub.H3 domain and

hinge regions of IgG1) or a COX-2 inhibitor such as celexicob (4-5[-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazole-1-yl] benzenesulfonamide) or rofecoxib (4-[4-methylsulfonyl)phenyl]-3-phenyl-2 (5H)-furanone), antimalarial agents, antimicrobials, antimigraine agents, antimycotic agents, antinausea agents, antineoplastic agents, antiparasitics, antiparkinsonian agents, antiproliferatives, antiprostatic hypertrophy agents, antiprotozoals, antipruritics, antipsychotics, antirheumatics, antischistosomals (e.g., praziquantel, artemisinin), blood glucose regulators, bone resorption inhibitors, bronchodilators, cardiac depressants, cardioprotectants, choleretics, depressants, diuretics, dopaminergic agents, enzyme inhibitors, free oxygen radical scavengers, glucocorticoids, peptide hormones, steroid hormones, hypocholesterolemics, hypoglycemics, hypolipidemics, hypotensives, immunomodulators, liver disorder treatments, mucosal protective agents, nasal decongestants, neuromuscular blocking agents, plasminogen activators, platelet activating factor antagonists, platelet aggregation inhibitors, post-stroke and post-head trauma treatments, progestins, psychotropics, radioactive agents, relaxants, sclerosing agents, sedatives, sedative-hypnotics, selective adenosine A1 antagonists, serotonin antagonists, serotonin inhibitors, serotonin receptor antagonists, thyroid inhibitors, thyromimetics, tranquilizers, vasoconstrictors, vasodilators, wound healing agents, xanthine oxidase inhibitors or a treatment(s) or therapeutic agent(s) for amyotrophic lateral sclerosis, ischemia, e.g., cereberal ischemia, cardiac ischemia or cardiovascular ischemia, or unstable angina. The selection and use of these agents for a particular subject will typically use dosing methods, dosages and routes of administration essentially according to known methods, dosages and routes of administration. Such methods, dosages and routes of administration are described in detail at, e.g., Textbook of Autoimmune Diseases, R. G. Lahita, editor, Lippincott Williams & Wikins, Philadelphia, Pa., 2000, ISBN 0-7817-1505-9, pages 81-851, Holland.cndot.Frei Cancer Medicine .sup.e.-5, 5.sup.th edition, R. C. Bast et al., editors, 2000, ISBN 1-55009-113-1, pages 168-2453, B. C. Becker Inc. Hamilton, Ontario, Canada, Hematology, Basic Principles and Practice, 3.sup.rd edition, R. Hoffman, et al., editors, 2000, ISBN 0-443-77954-4, pages 115-2519, Churchill Livingstone, Philadelphia, Pa., Rheumatology, 2.sup.nd edition, J. H. Klippel et al., editors, 1998, ISBN 0-7234-2405-5, volume 1, sections 1-5 and volume 2, sections 6-8, Mosby International, London, UK, Alzheimer's Disease and Related Disorders: Etiology, Pathogenesis and Therapeutics, K. Iqbal, et al., editors, 1999, ISBN 0-471986386, John Wiley & Son Ltd, and Cardiovascular Medicine, E. J. Topol, editor, Lippincott Williams & Wikins, Philadelphia, Pa., 1998, ISBN 0781716810.

Summary of Invention Paragraph:
[0777] An antigenic protein, peptide or glycoprotein can be identified by standard methods, e.g., protein or nucleic acid sequencing, for any of the infectious agents or tumors that are described herein or in the cited references. Thus, in some embodiments, an effective amount of a formula 1 compound and an antigen are administered to a subject, or delivered to the subject's tissues, to stimulate an immune response against the antigen. The antigen may comprise one, two or more antigenic epitopes, which may come from one, two or more genes. In some embodiments, the subject is optionally monitored to follow or determine the immune, dendritic cell, B cell, T cell, antibody or cytokine response, such as one disclosed herein, e.g., modulation or increase in .gamma.IFN, IL-2 or IL-12 levels or measurement of the production of one or more immunoglobulin types or subtypes. The subject may also be monitored by in vitro cell assays, e.g., for activation of T cells or subsets of T cells or other relevant white blood cell types. Such assays include measuring T cell activation using chromium release assays, or mixed lymphocyte assays. The subject is optionally treated with one or more additional booster vaccinations, when this is called for under the circumstances.

Summary of Invention Paragraph:
[0792] These conditions include cancers or precancers comprising carcinomas, sarcomas, adenomas, blastoma, disseminated tumors and solid tumors such as one associated with or arising from prostate, lung, breast, ovary, skin, stomach,

intestine, pancreas, neck, larynx, esophagus, throat, tongue, lip, oral cavity, oral mucosa, salivary gland, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, vagina, pelvis, endometrium, kidney, bladder, central nervous system, glial cell, astrocyte, squamous cell, blood, bone marrow, muscle or thyroid cells or tissue. The formula 1 compounds are thus useful to treat, prevent, slow the progression of, or ameliorate one or more symptoms of a precancer, cancer or related hyperproliferation condition such as myelodysplastic syndrome, actinic keratoses, endometriosis, Barrett's esophagus, leiomyoma, fibromyoma, benign or precancerous intestinal or bowel polyps or benign prostatic hyperplasia. The compounds can also be used to treat, prevent, slow the progression of, slow the replication or growth of, or to ameliorate one or more symptoms of a primary tumor, a metastasis, an advanced malignancy, a blood born malignancy, a leukemia or a lymphoma.

Summary of Invention Paragraph:
[0796] In some of these embodiments, the formula 1 compounds may be used to treat, prevent or slow the progression of or ameliorate one or more conditions in a subject having or subject to developing a hyperproliferation condition where angiogenesis contributes to the pathology. Abnormal or unwanted angiogenesis or neovascularization contributes to the development or progression of solid tumor growth and metastases, as well as to arthritis, some types of eye diseases such as diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, rubeosis, retinoblastoma, uvietis and pterygia or abnormal blood vessel growth of the eye, and psoriasis. See, e.g., Moses et al., Biotech. 9:630-634 1991, Folkman et al., N. Engl. J. Med., 333:1757-1763 1995, and Auerbach et al., J. Microvasc. Res. 29:401-4111985.

Summary of Invention Paragraph:
[0806] Exemplary symptoms that the use of the formula 1 compounds can ameliorate include one or more of pain such as arm, jaw or chest pain, edema or swelling, high blood pressure, shortness of breath or dyspnea, e.g., on exertion or while prone, fatigue or malaise and low cardiac injection fraction. In treating a cardiovascular condition in a subject or in improving one or more symptoms thereof, the formula 1 compounds may accomplish one or more of increasing cardiac ejection volume or fraction, decreasing levels of IL-6, decreasing levels of C reactive protein, fibrinogen, cardiac creatinine kinase, increasing fatty acid metabolism or utlization by cardiac tissue, increasing carnityl palmitoyl fatty acid transferase or other cardiac metabolic enzymes, activating potassium dependent calcium channels, vasodilating or enhancing oxygen delivery to ischemic tissues or decreasing levels of scarring or plaque formation that occurs, e.g., after vascular damage. Symptoms associated with a cardiovascular condition such as ischemia that can be ameliorated also include acidosis, expression of one or more immediate early genes in, e.g., glial cells, vascular smooth muscle cells or endothelial cells, neuronal membrane depolarization and increased neuronal extracellular calcium and glutamate concentration. Other biological effects associated with treatment using a formula 1 compound may also be monitored, e.g., and increase or decrease of a cell surface antigen, a cytokine or an interleukin as disclosed herein.

Summary of Invention Paragraph:
[0808] The formula 1 compounds also can limit inflammation or cell injury that is associated with ischemia or oxygen reperfusion after ischemia. lschemia, which is a detrimental decrease in oxygenated blood delivery to affected cells or tissues, may arise from a cardiovascular condition or event such as an infarction, or from thermal injury or burns. lschemia may also arise from accidental or surgical trauma. Reperfusion after cells have become hypoxic for a sufficient period of time can lead to tissue or cell injury that varies from slight to lethal. The compounds can reduce cell or tissue injury or death associated with ischemia and reperfusion, by, e.g., reducing inflammation or the level of a molecule associated with inflammation. Thus, levels of a proinflammatory cytokine or molecule such as leukotriene B4, platelet activating factor or levels of extracellular P-selectin

may result from administration of a formula 1 compound to a subject who may experience reperfusion injury. Thus, the compounds can reduce injury or death of, e.g., neuron, cardiac, vascular endothelium, myocardial, pulmonary, hepatic or renal cells or tissues. Without wishing to be bound by any theory, the compounds may act in part by reducing one or more of neutrophil activation, platelet activation, platelet aggregation, endothelial cell activation and neutrophil adherence or adhesion to endothelial cells in these conditions.

Summary of Invention Paragraph:
[0829] For any of these diseases, conditions or their associated symptoms, the presence of the disease, condition or symptom may be determined by suitable objective or subjective means, e.g., assays to detect tissue damage, levels of diagnostic markers, or an etiological agent, patient questionaires or behavior performance tests, measurement of a diagnostic marker(s), e.g., an enzyme, hormone, cytokine or drug substance in blood or tissue, electroencephalography, imaging methods such as X-ray, MRI scan or CAT scan, observation and diagnosis of clinical features or symptoms or biopsy of affected tissue or cells, e.g., aspiration biopsy, needle biopsy, incision biopsy or punch biopsy of tissue or cells. Neurological conditions, diseases and symptoms, which the formula 1 compounds can be used to treat or ameliorate and methods to diagnose and characterize such conditions or diseases have been described. See, e.g., Ph. Demaerel, A. L. Baert et al., eds. Recent Advances in Diagnostic Neuroradiology (Medical Radiology: Diagnostic Imaging) 2001 Springer Verlag, ISBN: 3504657231, W. G. Bradley et al., Neurology in Clinical Practice: Principles of Diagnosis and Management 1995, see, e.g., vol. 1 Ch. 1-55 and vol 2. Ch. 1-66, Butterworth-Heinemann Medical, ISBN 0750694777, H. J. M. Barnett et al., eds. Stroke: Pathophysiology, Diagnosis and Management 3.sup.rd edition, 1998, see, e.g., pages 10-1450, Churchill Livingstone, ISBN 0443075514, P. J. Vinken et al., eds. Neurodystrophies and Neurolipidoses 2.sup.nd ed. 1996, see, e.g., pages 8-780, Elsevier Science, ISBN 0444812857, P. L. Peterson and J. W. Phillis eds. Novel Therapies for CNS Injuries: Rationales and Results 1995, see, e.g., pages 8-380, CRC Press, ISBN 0849376521, D. Schiffer, Brain Tumors: Pathology and Its Biological Correlates 2.sup.nd ed. 1997, see, e.g., pages 5-450, Springer Verlag, ISBN 3540616225 and E. Niedermeyer and F. Lopes Da Silva, eds. Electroencephalography: Basic Principles, Clinical Applications and Related Fields 4.sup.th ed. 1999 see, e.g., pages 13-1238, Lippincott, Williams & Wilkins, ISBN 0683302841. The use of the formula 1 compounds in these conditions is optionally combined with one or more of the therapeutic treatments that are described in these references. The formula 1 compound may be administered before, during or after another treatment is employed to treat or ameliorate a given neurological disease, condition or symptom.

Summary of Invention Paragraph:
[0835] The invention includes methods to treat or prevent various blood cell deficiencies such as TP or NP. Without being bound to any theory, the treatment methods may at least in part result in enhanced hematopoiesis (or hemopoiesis) or the treatment methods may reduce the loss of cells such as platelets or neutrophils. Increased platelet or neutrophil production or reduced loss is typically observed as increased circulating blood cell counts. Thus, invention aspects comprise methods to treat or prevent neutropenia in a subject in need thereof, comprising administering to a subject in need, or delivering to the subject's tissues, an effective amount of a formula 1 compound.

Summary of Invention Paragraph:
[0836] Normal ranges of various white blood cells or blood components in adult (about 18-49 years of age) human blood are as follows. Total adult white blood cell counts average about 7500/mm.sup.3, with an approximate normal range of about 4.5-11.0.times.10.sup.3/mm.sup.3. The normal basophil level is about 35 mm.sup.-3, with a normal range of about 10-100/mm.sup.3. The normal adult neutrophil level is about 4400/mm.sup.3, with a normal range of about 2000-7700/mm.sup.-3. The normal eosinophil level is about 275 mm.sup.-3, with a normal range of about 150-

300/mm.sup.3. The normal monocyte level is about 540 mm.sup.-3, with a normal range of about 300-600/mm.sup.3. The normal adult platelet level is about 2.5.times.10.sup.5/mm.sup.3, with a normal range of about 2.1.times.10.sup.5-2.9.times.10.sup.5/mm.sup.3. The normal adult red cell mass corresponds to about 4.6.times.10.sup.12 red cells/L in females and about 5.2.times.10.sup.12 red cells/L in males.

Summary of Invention Paragraph:
[0838] In some cases, the diagnosis of a deficiency may cover a cell count that falls outside these ranges, due, e.g., to individual variations in a subject's age, sex, race, animal strain or normal blood cell status for the individual. Such variations are identified by known means such as by identification of a change from the subject's normal status or by multiple cell measurements over time that reveal a deficiency. See, e.g., Hematology--Basic Principles and Practice, 2.sup.nd edition, R. Hoffman, E. J. Benz Jr. et al., editors, Churchill Livingstone, New York, 1995. Subjects with an identified or identifiable deficiency outside these standard ranges are included in the definition of a blood cell deficiency or a subject in need of treatment, as used herein.

Summary of Invention Paragraph:
[0839] Specific conditions that are amenable to prophylaxis or treatment by the invention methods include the acquired blood cell deficiencies. Exemplary deficiencies or groups of deficiencies are neonatal alloimmune TP, immune TP, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, post-transfusion purpura, radiation associated TP, chemotherapy associated TP (e.g., NSAID treatments such as with indomethicin, ibuprofen, naproxen, phenylbutazone, piroxicam or zompirac, or .beta.-lactam antibiotic treatments such as with ampicillin, carbenicillin, penicillin G, ticarcillin, or cephalosporin treatments such as with cefazolin, cefoxitin or cephalothin, anticoagulant treatments such as heparin, hirudin, lepirudin or aspirin, treatment with plasma expanders or psychotropic drugs), amegakaryocitic TP, chemotherapy associated TP, radiation associated TP, TP associated with solid organ allograft or xenograft rejection or immune suppression therapy in solid organ or other tissue transplants (e.g., liver, lung, kidney, heart, bone marrow, hematopoietic stem cell or endothelial cell transplant, implant or transfusion), cardiopulmonary bypass surgery or chemotherapy associated TP (e.g., an anticancer, antiviral, antibacterial, antifungal or antiparasite therapy), cardiovascular disease or therapy associated TP (e.g., congenital cyanotic heart disease, valvular heart disease, pulmonary embolism, pulmonary hypertension disorders or diltiazem, nifedipine, nitroglycerin or nitroprusside therapy), TP associated with chronic or acute renal failure or treatment for these conditions (e.g., dialysis), TP associated with infection such as a virus or bacterial infection, postinfectious NP, drug-induced NP, autoimmune NP, chronic idiopathic NP, basophilic leukopenia, eosinophilic leukopenia, monocytic leukopenia, neutrophilic leukopenia, cyclic NP, periodic NP, chemotherapy associated NP, radiation associated NP, chemotherapy associated NP, radiation associated NP, NP associated with solid organ allograft or xenograft rejection or immune suppression therapy in solid organ or other tissue transplants (e.g., liver, lung, kidney, heart, bone marrow, hematopoietic stem cell or endothelial cell transplant, implant or transfusion), chemotherapy associated leukopenia, radiation associated leukopenia, leukopenia associated with solid organ allograft or xenograft rejection or immune suppression therapy in solid organ or other tissue transplants (e.g., liver, lung, kidney, heart, bone marrow, hematopoietic stem cell or endothelial cell transplant, implant or transfusion), immune hemolytic anemias, anemia associated with chronic or acute renal failure or treatment for these conditions (e.g., dialysis), anemia associated with chemotherapy (e.g., isoniazid, prednisone) or anemia associated with radiation therapy.

Summary of Invention Paragraph:
[0840] Some of the blood cell deficiencies are associated with, or caused by, other therapeutic treatments, e.g., cancer chemotherapy, anti-pathogen chemotherapy,

radiation therapy and chemotherapy for suppression of autoimmunity or immune suppression therapy for organ or tissue transplantation or implantation. The formula 1 compounds are thus useful to facilitate or speed up immune system recovery in autologous bone marrow transplant or stem cell transplant situations. In many cases it would be medically sound to continue the treatment associated with causing or exacerbating the blood cell deficiency. Thus, one would generally conduct the invention methods with subjects who are undergoing another therapy at the same time or near the same time, e.g., within a few days to within about 1-6 months. Such subjects typically will have an identified blood cell deficiency such as a NP or a TP, e.g., as disclosed herein. However, the formula 1 compounds are generally suitable for preventing the onset of such deficiencies, and they can thus be used prophylactically in these indications. The invention includes all of these embodiments.

Summary of Invention Paragraph:
[0841] In some embodiments, the invention method is accomplished using an effective amount of one or more growth factors or cytokines as a means to further enhance the effect of the formula 1 compounds for their intended uses or to modulate their effects. Suitable growth factors and cytokines are as described herein or in the cited references. For example, when one administers the formula 1 compound to enhance generation of platelets in humans or other subjects, or their precursor cells such as CFU-GEMM, BFU-Mk, CFU-Mk, immature megakaryocytes or mature postmitotic megakaryocytes, one can also administer one or more of G-CSF, GM-CSF, SCF, Steel factor ("SF"), leukemia inhibitory factor ("LIF"), interkeukin-1.alpha., ("IL-1.alpha."), IL-3, IL-6, IL-11, TPO, EPO, their isoforms, their derivatives (e.g., linked to a PEG or fusions such as PIXY321) or their homologs for other species. Similarly, administration of the formula 1 compound to enhance the generation or function of myelomonocytic cells such as neutrophils, basophils or monocytes in humans or other subjects, one can also administer one or more of G-CSF, GM-CSF, M-CSF, LIF, TPO, SF, interleukin-1 ("IL-1"), IL-2, IL-3, IL-4, interleukin-5 ("IL-5"), IL-6, IL-11, interleukin-12 ("IL-12"), interleukin-13 ("IL-13"), FLT3 ligand, their isoforms, homologs or derivatives (e.g., linked to a PEG or fusions such as PIXY321) or their homologs for other species. To enhance generation of red cells or their precursor cells such as CFU-GEMM, BFU-E or CFU-E in humans being treated with a formula 1 compound, one can co-administer one or more of G-CSF, GM-CSF, IL-1, IL-3, IL-6, TPO, EPO, transforming growth factor-1, their isoforms, their derivatives (e.g., linked to a PEG or fusions such as PIXY321) or their homologs for other species. See, e.g., Hematology--Basic Principles and Practice, 3.sup.rd edition, R. Hoffman, E. J. Benz Jr. et al., editors, Churchill Livingstone, New York, 2000 (see, e.g., Chapters 14-17 at pages 154-260). The co-administration of such factors in these methods is intended to enhance the efficacy of the formula 1 compound treatment, which is optionally measured by taking suitable blood or tissue, e.g., bone marrow, samples at one or more times before and after the compounds have been administered. Such co-administration will generally be compatible with a subject's condition and other therapeutic treatments. Co-administration of such factors can precede, be simultaneous with, or follow the times of administration of the formula 1 compound (s) to the subject. Dosages of such growth factors would generally be similar to those previously described, e.g., typically an initial course of treatment comprises administering about 1.0 to about 20 .mu.g/kg/d for about 1-10 days, or as described in, e.g., Hematology--Basic Principles and Practice, 3.sup.rd edition, R. Hoffman, E. J. Benz Jr. et al., editors, Churchill Livingstone, New York, 2000 (see, e.g., Chapter 51 at pages 939-979 and the references cited therein).

Summary of Invention Paragraph:
[0842] In cases where a subject's blood cell deficiency is caused by, or associated with another therapy, the invention contemplates that the other therapy will continue, if this is reasonable under the circumstances. The timing of other therapies can precede, be simultaneous with, or follow the times of administration of the formula 1 compound(s) to the subject. For example, chemotherapy for some

malignancies is accompanied by myelosuppression or a deficiency in one or more blood cell types, e.g., TP or NP. Continued treatment would be called for in some cases, and then the invention methods would be employed to deliver to the subject an effective amount of a formula 1 compound. Thus, alkylating agents, antimicrotubule agents, antimetabolites, topoisomerase 1 or 11 inhibitors, or platinum compounds such as one or more of mechlorethamine, vincristine, vinblastine, bleomycin, doxorubicin, epirubicin, tamoxifen, cyclophosphamide, etoposide, methotrexate, ifosfamide, melphalan, chlorambucil, busulfan, carmustine, lomustine, streptozocin, dacarbazine, vinorelbine, paclitaxel (taxol), docetaxel, cytosine arabinoside, hydroxyurea, fludarabine, 2'-chlorodeoxyadenosine, 2'-deoxycoformycin, 6-thioguanine, 6-mercaptopurine, 5-azacytidine, gemcitabine, arabinofuranosylguanine, daunorubicin, mitoxantrone, amsacrine, topotecan, irinotecan, cisplatin, carboplatin, pilcamycin, procarbazine, aspariginase, aminoglutethimide, actinomycin D, azathioprine and gallium nitrate may be administered in conjunction with administration of any formula 1 compound(s) that is disclosed herein. Treatments with other therapeutic agents such as heparin or nucleoside analogs such as 3-thiacytosine, azidothymidine or dideoxycytosine, or other antimicrobials such as cephalosporin, quinine, quinidine, gold salts (e.g., aurothioglucose), a fluoroquinolone (e.g., ciprofloxacin), clarithromycin, fluconazole, fusidic acid, gentamycin, nalidixic acid, penicillins, pentamidine, rifampicin, sulfa antibiotics, suramin or vancomycin may result in a blood cell deficiency(s) and they can thus be combined with administration of a formula 1 compound to treat the deficiency, or to ameliorate a symptom thereof. Similarly, anti-inflammatory drugs (e.g., salicylates, entanercept (a dimeric fusion coprising a portion of the human TNF receptor linked to the Fc protion of human IgG1 containing the C.sub.H2 and C.sub.H3 domain and hinge regions of IgG1) or a COX-2 inhibitor such as celexicob (4-5[-(4-methylphenyl)-3-- (trifluoromethyl)-1H-pyrazole-1-yl] benzenesulfonamide) or rofecoxib (4-[4-methylsulfonyl)phenyl]-3-phenyl-2 (5H)-furanone) or an IL-1 receptor antagonist such as anakinra), cardiac drugs (e.g., digitoxin), .beta.-blockers or antihypertensive drugs (e.g., oxprenolol or captopril), diuretics (e.g., spironolactone), benzodiazepines, (e.g., diazepam) or antidepressants (e.g., amitriptyline, doxepin). Any of these methods also optionally include co-administration of one or more of the growth factors described above, e.g., IL-3, G-CSF, GM-CSF or TPO.

Summary of Invention Paragraph:
[0846] In conducting any of these methods, one can monitor the subject's clinical condition at any relevant time before, during or after administration of the formula 1 compounds, which treatments are optionally combined with any of the other agents or treatments disclosed herein, such as cytokines, interleukins or an agent or molecule that can stimulate the activity or number of neutrophils or monocytes. The subject's blood can be drawn on one, two or more occasions in advance of treatment to, e.g., obtain a baseline or initial level of white or red blood cells, to verify a presumptive diagnosis of a blood cell deficiency or to determine a blood parameter such as circulating myelomonocyte counts, circulating neutrophil counts, circulating platelet counts or the myeloperoxidase index. Then, during the course of treatment or thereafter the subject's blood can be drawn on one, two or more occasions to follow the subject's response.

Summary of Invention Paragraph:
[0847] Invention embodiments include methods that comprise administering to a subject in need thereof an effective amount of a formula 1 compound and an effective amount of at least one form of interferon, such as y-Interferon or a growth factor or interleukin such as G-CSF or IL-6. Interferons can enhance the biological activity of the white cells that arise from increased hemopoiesis. This can be particularly useful when the subject's circulating blood cell deficiency is associated with, e.g., an infection or a chemotherapy that suppresses hemopoiesis. Administration of an growth factor or an interleukin such as IL-6 can facilitate hemopoiesis by stimulating quiescent stem cells or other progenitors that give rise to deficient cell types. Related embodiments replace growth factor or interferon

administration partially or completely by increasing endogenous production in the subject using conventional methods, e.g., administering double stranded RNA to stimulate .gamma.-IFN.

Summary of Invention Paragraph:
[0850] An aspect of the invention is method to enhance hemopoiesis in a subject in need thereof comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a compound of formula 1. In some embodiments, the formula 1 compound is not 5-androstene-3.beta.-ol-17-one, 5-androstene-3.beta.,17.beta.-diol, 5-androstene-3.beta.,7.beta.,17.beta.-triol or a derivative of any of these three compounds that can convert to these compounds by hydrolysis. Exemplary formula 1 compounds in this method include compounds wherein (1) one or two R.sup.10 at the 1, 4, 6, 8, 9, 12 and 14 positions is not --H, wherein the one or two R.sup.10 at the 1, 4, 6, 8, 9, 12 and 14 positions are independently selected from --F, --Cl, --Br, --I, --OH, .dbd.O, --CH.sub.3, --C.sub.2H.sub.5, an ether optionally selected from --OCH.sub.3 and --OC.sub.2H.sub.5, and an ester optionally selected from --O--C(O)--CH.sub.3 and --O--C(O)--C.sub.2H.sub.5, and/or (2) R.sup.1, R.sup.2, R.sup.3 and R.sup.4 are independently selected from --H, --OH, .dbd.O, an ester and an ether. In these embodiments, the subject may have thrombocytopenia or neutropenia or the subject's circulating platelets, red cells, mature myelomonocytic cells, or their precursor cells, in circulation or in tissue may be detectably increased. In some cases the subject has renal failure. These methods may further comprise the steps of obtaining blood from the subject before administration of the formula 1 compound and measuring the subject's white or red cell counts and optionally, on one, two, three or more occasions, measuring the subject's circulating white cell or red cell counts after administration of the formula 1 compound, e.g., within about 12 weeks after an initial administration of a formula 1 compound or during or within about 12 weeks after a course of treatment. Such a treatment course may as described herein.

Summary of Invention Paragraph:
[0852] For administration of .gamma.-IFN, a volume of about 1 mL of a solid or liquid sublingual formulation that comprises about 100 micrograms of .gamma.-IFN may be used. An exemplary liquid formulation comprises a saline solution containing 45 weight % .beta.-hydroxypropylcyclodextrin. It would be expected that such a dosage would provide in the range of 30 to 40 micrograms of .gamma.-IFN to the patient's blood. Such sublingual formulations would be held under the patient's tongue for a period of time sufficient to allow some or all of the .gamma.-IFN to be delivered to the patient while held under the patient's tongue. Such administration has not been previously known in the art, in which conventionally, it has been thought that administration of .gamma.-IFN must be by injection, e.g., subcutaneous injection. Subcutaneous injection of .gamma.-IFN is associated with unwanted side effects, including fatigue, headache, night sweats, fever, local pain at the injection site, nausea, vomiting, diarrhea and others. The above-described sublingual .gamma.-IFN formulations of the present invention is an aspect of the present invention, which can be of use in accordance with other aspects of the present invention as described herein. In general, however, a wide variety of routes of administration could be employed for .gamma.-IFN in accordance with the present invention, including those disclosed in U.S. Pat. No. 5,145,677.

Summary of Invention Paragraph:
[0855] In many of the clinical conditions described herein, e.g., in cancers, infections, acute inflammation, chronic inflammation or autoimmunity, the formula 1 compounds can modulate, e.g., detectably decrease or increase, a biological activity(ies), protein or molecule level or RNA level of 1, 2, 3, 4, 5, 6 or more biomolecules that are involved in establishment, maintenance or progression of a disease, condition or symptom. Such biomolecules include 1, 2, 3, 4, 5, 6 or more of AP-1, a qyclooxygenase such as cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2), TNF.alpha., TNF.alpha. receptor 1, TNF.alpha. receptor 2, TNF receptor-

associated factor, TNF.beta., TNF.beta. receptor, MIP-1.alpha., monocyte chemoattractant-1 (MCP-1), interferon gamma (IFN.gamma. or .gamma.IFN), IL-1.alpha., IL-1.beta., IL-1.alpha. receptor, IL-1.beta. receptor, IL-2, IL-3, IL-4, IL-4 receptor (IL-4R), IL-5, IL-6, IL-6 receptor (IL-6R), IL-8, IL-8 receptor (IL-8R), IL-10, IL-10 receptor (IL-10R), IL-12, an IL-12 receptor, (e.g., IL-12R.beta.2), IL-13, IL-15, IL-17, IL-18, nuclear factor kappa B (NF.kappa.B), AP-1, c-maf, v-maf, mafB, Nrl, mafK, mafG, the maf family protein p18, reactive oxygen species, e.g., hydrogen peroxide or superoxide ion (collectively ROS), a 17.beta.-hydroxysteroid dehydrogenase (17.beta.-HSD) or an 11.beta.-hydroxysteroid dehydrogenase (11.beta.-HSD), e.g., 11.beta.-HSD type 1,11.beta.-HSD type 2, 17.beta.-HSD type 1, 17.beta.-HSD type 2 or 17.beta.-HSD type 5, a steroid aromatase, e.g., cytochrome P450 aromatase, steroid 5.alpha.-reductase, serum or blood cortisol cytosolic phospholipase A2 (cPLA2), calcium-independent phospholipase A2 (iPLA2), a prostaglandin, e.g., prostaglandin E2 (PGE2) or prostaglandin D2 (PGD2), a leukotriene, e.g., leukotriene B4, inducible nitric oxide synthetase (iNOS), nitric oxide (NO), GM-CSF, RANTES (regulated on activation, normal T cells expressed and secreted), eotaxin, GATA-3, CCR1, CCR3, CCR4, CCR5, CXCR4,, in, e.g., a subject's cell(s) or tissue(s) or in enzyme, tissue or cell-based assays. In these subjects, the levels of other biomolecules, their RNAs or the level of their activity can be detectably modulated include IFN.alpha., INF.alpha. receptor, PPAR.alpha., PPAR.gamma., PPAR.delta. or a transcription factor such as T-bet is detectably increased. Other biomolecules or their polymorphs or homologs that the formula 1 compounds directly or indirectly modulate include one or more of, e.g., Janus kinase 1 (JAK1), Janus kinase 2 (JAK2), Janus kinase 3 (JAK3), signal transducer and activator of transcription 1 (STAT1), signal transducer and activator of transcription 2 (STAT2) and signal transducer and activator of transcription 3 (STAT3). The formula 1 compounds can modulate the other biologically active analogs of any these enzymes, chemokines, cytokines, their receptors or ligands, including their polymorphs or homologs. In some cells or tissues, one or more of these biomolecules may be detectably increased, while in other cells or tissues, the same biomolecule may be detectably decreased. Thus, the biomolecules that the formula 1 compounds can modulate, e.g., detectably increase or decrease, include the intracellular or extracellular level or biological activity of one or more enzyme, cytokine, cytokine receptor, chemokine and/or chemokine receptor.

Summary of Invention Paragraph:

[0884] The formula 1 compounds can directly or indirectly modulate the activity or synthesis of one or more biological ligands to effect a detectable biological response or activity change. To facilitate the identification of candidate binding partners for the formula 1 compounds, one can use a radiolabeled formula 1 compound that is linked to a support, usually a solid support, as a means to recover the candidate binding partners. The formula 1 compound can be linked to the support through a variable group that is bonded to the formula 1 compound, e.g., at the 2-, 3-, 7-, 11-, 15-, 16- or 17-position of the steroid nucleus. Linking agents are known for such uses and include homobifunctional and heterobifunctional agents, many of which are commercially available. The linker one uses will typically comprise about 2-20 linked atoms. The linked atoms usually comprise mostly carbon, with one, two or three oxygen, sulfur or nitrogen atoms that optionally replace one or more carbon or hydrogen atoms. One can use a cDNA expression library that one has made from suitable cells or tissues as a source of candidate binding partners. The cells or tissues can be obtained from a mammalian or a vertebrate host, e.g., human, mouse, bird, primate, or from other sources, e.g., insects (e.g., Drosophila), other invertebrates (e.g., yeast, bacteria, Mycoplasma sp., Plasmodium sp., Tetrahymena sp., C. elegans) or other organism groups or species listed herein or in the cited references. Suitable tissues include skin, liver tissue or cells, including hepatocytes and Kupfer cells, fibrocytes, monocytes, dendritic cells, kidney cells and tissues, brain or other central nervous system cells or tissues, including neurons, astrocytes and glial cells, peripheral nervous system tissues, lung, intestine, placenta, breast, ovary, testes, muscle, including heart or

myocyte tissue or cells, white blood cells, including T cells, B cells, bone marrow cells and tissues, lymph tissues or fluids and chondrocytes.

Summary of Invention Paragraph:
[0886] Embodiments of the invention include a composition comprising a partially purified (purified at least about 2-fold to about 10-fold relative to natural sources, e.g., cells or a cell lysate) complex or a purified (purified at least about 20-fold to about 5000-fold relative to natural sources, e.g., cells or a cell lysate) complex (where the partially purified or purified complex is optionally isolated) comprising a formula 1 compound and a steroid receptor, a serum steroid-binding protein (e.g., human serum albumin, p1-acid glycoprotein, sex hormone-binding globulin, testosterone-binding globulin, corticosteroid-binding globulin, androgen binding protein (rat)) or another binding partner, e.g., transcription factor or DNARS. An aspect of these compositions includes a product produced by the process of contacting the partially purified or the purified composition with one or more cells, one or more tissues, plasma or blood.

Summary of Invention Paragraph:
[0890] Methods suitable to measure the cellular response or biological effects caused by various compounds, e.g., activation, on immune system cells (e.g., NK cells, phagocytes, monocytes, macrophage, neutrophils, eosinophils, dendritic cells, synoviocytes, microglial cells, fibrocytes) have been described, e.g., Jakob et al., J. Immunol. 1998 161:3042-3049, Pierson et al., Blood 1996 87:180-189, Cash et al., Clin. Exp. Immunol. 1994 98:313-318, Monick et al., J. Immunol. 1999 162:3005-3012, Rosen et al., Infect. Immun. 1999 67:1180-1186, Grunfeld et al., J. Lipid Res. 1999 40:245-252, Singh et al., Immunol Cell Biol. 1998 76:513-519, Chesney et al., Proc. Natl. Acad. Sci. USA 1997 94:6307-6312, Verhasselt et al., J. Immunol. 1999 162:2569-2574, Avice et al., J. Immunol. 1999 162:2748-2753, Celia et al., J. Exp. Med. 1999 189:821-829, Rutalt et al., Free Radical Biol. Med. 1999 26:232-238, Akbari et al., J. Exp. Med. 1999 189:169-178, Hryhorenko et al., Immunopharmacology 1998 40:231-240, Fernvik et al., Inflamm. Res. 1999 48:28-35, Cooper et al., J. Infect. Dis. 1999 179:738-742, Betsuyaku et al., J. Clin. Invest. 1999 103:825-832, Brown et al., Toxicol. Sci. 1998 46:308-316, Sibelius et al., Infect Immunol. 1999 67:1125-1130. The use of formula 1 compounds in such methods are aspects of the invention and they permit, e.g., measurement of the biological effects of formula 1 compounds on, e.g., one or more of (1) the cell's biological activities, (2) genes whose expression is regulated by the formula 1 compound or (3) a steroid receptor. Exemplary biological effects that the formula 1 compounds may exert include one or more of (1) stimulation of ion flux or ion channel activity in one or more immune cell subsets such as one or more of those described herein, (2) binding to one or more ligands such as a steroid receptor and modulation of a biological activity of the receptor, (3) detectably enhanced transcription of one or more genes whose expression is affected by a steroid receptor(s) or other biomolecule whose activity is directly or indirectly affected by the formula 1 compound's presence and (4) detectably decreased transcription of one or more genes whose expression is affected by a steroid receptor(s) or other biomolecule whose activity is directly or indirectly affected by the formula 1 compound's presence.

Summary of Invention Paragraph:
[0893] Other related embodiments are a composition comprising a partially purified or a purified complex comprising a formula 1 compound and a steroid receptor, a serum steroid-binding protein (e.g., human serum albumin, .alpha.1-acid glycoprotein, sex hormone-binding globulin, testosterone-binding globulin, corticosteroid-binding globulin, androgen binding protein (rat) or a homolog or isoform of any of these) or another binding partner, e.g., transcription factor or DNARS. An aspect of these compositions includes a product produced by the process of contacting the partially purified or the purified composition with one or more cells, one or more tissues, plasma or blood.

Summary of Invention Paragraph:
[0894] In a related embodiment, a formula 1 compound is used to exert a cytostatic effect on a subject's cells, e.g., mammalian cells, in vitro or in vivo. Typically such cells are lymphoid cells, e.g., T cell populations from, e.g., blood or organs that are rich in lymphoid cells (e.g., spleen, lymph tissue or nodes), or transformed T cell lines. Such activity provides an estimate of the potency of formula 1 compounds to mediate immunological effects, such as enhancing Th1 immune responses or suppressing expression of one or more Th2-associated cytokines. Thus, an invention method comprises (a) contacting a formula 1 compound and lymphoid cells in vitro, (b) determining the degree of cytostasis that the compound exerts to identify a cytostatic compound and (c) optionally administering the cytostatic compound to an immune suppressed subject to determine the effect of the compound on one or more of the subject's immune responses as described herein, e.g., enhanced Th1 cytokine or cell response or decreased Th2-associated cytokine expression. Typically, such methods are conducted using a range of formula 1 compound concentrations and suitable controls, such as a known cytostatic agent or a blank that contains solvent that lacks the formula 1 compound. Inhibition of cell proliferation is measured by standard methods. Methods to measure the cytostatic effects of the compounds includes measuring viable cell numbers in treated and untreated cultures or by measuring DNA synthesis using e.g., .sup.3H-thymidine incorporation into DNA in treated and untreated cultures. Typical ranges of formula 1 concentrations in the cell growth medium are about 0.1 .mu.M to about 100 .mu.M, using about 4-6 different concentrations of compounds with a fixed number of cells (e.g., about 0.4.times.10.sup.5 to about 5.times.10.sup.5). The formula 1 compound is left in contact with the cells in tissue culture for a sufficient time to observe cytostasis, e.g., about 16 hours to about 6 days, typically about 24-72 hours. In these embodiments, one may optionally screen for modulation of a biological activity of a steroid receptor, e.g., activation of PPAR.alpha., which may be associated with the cytostasis the compound induced.

Summary of Invention Paragraph:
[0898] The formula 1 compounds are useful in treating insulin resistance and associated symptoms and conditions. Insulin resistance is typically observed as a diminished ability of insulin to exert its biological action across a broad range of concentrations. This leads to less than the expected biologic effect for a given level of insulin. Insulin resistant subjects or human have a diminished ability to properly metabolize glucose or fatty acids and respond poorly, if at all, to insulin therapy. Manifestations of insulin resistance include insufficient insulin activation of glucose uptake, oxidation and storage in muscle and inadequate insulin repression of lipolysis in adipose tissue and of glucose production and secretion in liver. Insulin resistance can cause or contribute to polycystic ovarian syndrome, impaired glucose tolerance, gestational diabetes, hypertension, obesity, atherosclerosis and a variety of other disorders. Insulin resistant individuals can progress to a diabetic state. The compounds can also be used in the treatment or amelioration of one or more condition associated with insulin resistance or glucose intolerance including an increase in plasma triglycerides and a decrease in high-density lipoprotein cholesterol, high blood pressure, hyperuricemia, smaller denser low-density lipoprotein particles, and higher circulating levels of plasminogen activator inhibitor-1. Such diseases and symptoms have been described, see, e.g., G. M. Reaven, J. Basic Clin. Phys. Pharm. 1998, 9: 387-406, G. M. Reaven, Physiol. Rev. 1995, 75: 473-486 and J. Flier, J. Ann. Rev. Med. 1983, 34:145-60.

Summary of Invention Paragraph:
[0899] The compounds can thus be used in diabetes, obesity, hyperlipidemia or hypercholesterolemia conditions to reduce body fat mass, increase muscle mass or to lower one or more of serum or blood low density lipoprotein, triglyceride, cholesterol, apolipoprotein B, free fatty acid or very low density lipoprotein compared to a subject that would otherwise be considered normal for one or more of these characteristics. These beneficial effects are typically obtained with with

little or no effect on serum or blood high density lipoprotein levels. The formula 1 compounds are useful to reduce or slow the rate of myocardial tissue or myocyte damage, e.g., fibrosis, or to enhance cardiac fatty acid metabolism in conditions, such as inflammation, where fatty acid metabolism is depressed or decreased. Elevated cholesterol levels are often associated with a number of other disease states, including coronary artery disease, angina pectoris, carotid artery disease, strokes, cerebral arteriosclerosis, and xanthoma, which the formula 1 compounds can ameliorate or slow the progression or severity of. Abnormal lipid and cholseterol conditions that can be treated include exogenous hypertriglyceridemia, familial hypercholesterolemia, polygenic hypercholesterolemia, biliary cirrhosis, familial combined hyperlipidemia, dysbetalipoproteinemia, endogenous hypertriglyceridemia, mixed hypertriglyceridemia and hyperlipidemia or hypertriglycidemia secondary to alcohol consumption, diabetic lipemia, nephrosis or drug treatments, e.g., corticosteroid, estrogen, colestipol, cholestyramine or retinoid treatments. Dosages, routes of administration and dosing protocols for the formula 1 compounds are essenitally as described herein. Where the condition is chronic, the formula 1 compounds will generally be administered to a subject such as a human for a relatively long time period, e.g., for about 3 months to about 10 years or more. Dosages, routes of administration and dosing protocols for the formula 1 compounds are essenitally as described herein. Dosing of the compound can be daily or intermittent using a dosing protocol using dosages as described herein, e.g., about 0.01 to about 20 mg/kg of a formula 1 compound administered to a subject once or twice per day daily or intermittently. The use of the formula 1 compounds can be combined with other suitable treatments, e.g., diet control or HMG-CoA reductase inhibitors such as Simvastatin.TM., Pravastatin.TM., Mevastatin.TM. or Lovastatin.TM..

Summary of Invention Paragraph:
[0904] As noted above, in some embodiments a treatment with a formula 1 compound is combined with a corticosteroid or glucocorticoid. Corticosteroids are used in a number of clinical situations to, e.g., decrease the intensity or frequency of flares or episodes of inflammation or autoimmune reactions in conditions such as acute or chronic rheumatoid arthritis, acute or chronic osteoarthritis, ulcerative colitis, acute or chronic asthma, bronchial asthma, psoriasis, systemic lupus erythematosus, hepatitis, pulmonary fibrosis, type I diabetes, type II diabetes or cachexia. However, many corticosteroids have significant side effects or toxicities that can limit their use or efficacy. The formula 1 compounds are useful to counteract such side effects or toxicities without negating all of the desired therapeutic capacity of the corticosteroid. This allows the continued use, or a modified dosage of the corticosteroid, e.g., an increased dosage, without an intensification of the side effects or toxicities or a decreased corticosteroid dosage. The side-effects or toxicities that can be treated, prevented, ameliorated or reduced include one or more of bone loss, reduced bone growth, enhanced bone resorption, osteoporosis, immunosuppression, increased susceptibility to infection, mood or personality changes, depression, headache, vertigo, high blood pressure or hypertension, muscle weakness, fatigue, nausea, malaise, peptic ulcers, pancreatitis, thin or fragile skin, growth suppression in children or preadult subjects, thromboembolism, cataracts, and edema. Dosages, routes of administration and dosing protocols for the formula 1 compound would be essentially as described herein. An exemplary dose of formula 1 compound of about 0.5 to about 20 mg/kg/day is administered during the period during which a corticosteroid is administered and optionally over a period of about 1 week to about 6 months or more after dosing with the corticosteroid has ended. The corticosteroids are administered essentially using known dosages, routes of administration and dosing protocols, see, e.g., Physicians Desk Reference 54.sup.th edition, 2000, pages 323-2781, ISBN 1-56363-330-2, Medical Economics Co., Inc., Montvale, N.J. However, the dosage of the corticosteroid may optionally be adjusted, e.g., increased about 10% to about 300% above the normal dosage, without a corresponding increase in all of the side effects or toxicities associated with the corticosteroid. Such increases would be made incrementally over a sufficient time period and as appropriate for the

subject's clinical condition, e.g., daily corticosteroid dose increases of about 10% to about 20% to a maximum of about 300% over about 2 weeks to about 1 year.

Summary of Invention Paragraph:
[0911] Other desirable modulation effects of the formula 1 compounds on cells or tissues include (1) inhibition of one or more of bone resorption or calcium release or gp80, gp130, tumor necrosis factor (TNF), osteoclast differentiation factor (RANKL/ODF), RANKL/ODF receptor, IL-6 or IL-6 receptor expression or biological activity in, e.g., bone loss or osteoporosis conditions or in osteoclasts, or in cancers such as prostate cancer, metastatic breast cancer or metastatic lung cancer (e.g., with bone metastases), (2) inhibition of osteoclastogenesis or osteoclast development from progenitor cells, (3) enhancement of NF.kappa.B inhibition that is mediated by steroid receptors, e.g., enhanced inhibition of estrogen receptor-.alpha. or estrogen receptor-mediated inhibition of NF.kappa.B in inflammation, rheumatoid arthritis or osteoporosis, (4) enhancement of osteoblastogenesis, osteoblast, bone callus or bone development, e.g., from progenitor cells in bone fractures, depressed bone healing situations (e.g., in a burn patient or in a patient being treated with a glucocorticoid), bone growth or osteoporosis or other bone loss conditions, by, e.g., modulation or enhancement of osteoblast replication or development or modulation or enhancement of the synthesis or biological activity of a transcription factor such as Cbfa1, RUNX2 or AML-3 (5) normalization of hypothalamic-pituitary-adrenal axis function in conditions where there is dysregulation such as in chronic inflammatory diseases, chronic asthma or rheumatoid arthritis (increased cortisol to ACTH ratio), (6) modulation of ligand-gated ion channels in neurons in, e.g., depression, sleep or memory disorders, (8) modulation of G-protein coupled receptors in neurons in, e.g., depression, sleep or memory disorders, (9) modulation, e.g., induction, of the synthesis or biological activity of metabolic enzymes such as a cytochrome (e.g., a CYP enzyme such as CYP1A1, CYP2B1, CYP2B10, CYP4A, CYP7A, CYP7A1, CYP7B, CYP7B1, P450 3A4, P450c17, P450scc, P450c21 or an isozyme, homolog or mutant of any of these) in cells or tissues such as liver cells, neurons, neuron precursor cells, brain, breast, testes or colon, (10) enhancement of collagen synthesis or levels in, e.g., skin in aging or skin damage from, e.g., trauma, thermal injury or solar radiation, (11) inhibition of nitric oxide production in cells or tissue, e.g., in nervous system tissue or in microglial cells in dementias such as Alzheimer's disease, (12) enhancing glucose-stimulated insulin synthesis in hyperglycemia or diabetes conditions, (13) modulation of gamma-aminobutyric acid (GABA), dopamine or N-methyl-D-aspartate (NMDA) receptor activity or levels in, e.g., brain tissue or neurons, (e.g., decreased GABA-mediated chloride currents or potentiation of neuronal response to NMDA in the hippocampus) in, e.g., conditions such as a dementia (Alzheimer's Disease), depression, anxiety, schizophrenia or memory loss due to, e.g., aging or another condition described herein, (14) modulating (e.g., enhancing) the expression or activity of a transcription factor(s), or a homolog(s) or isoform(s), such as SET, nerve growth factor inducible protein B, StF-IT, SF-1 in cells or tissues such as nerve cells, neuronal precursor cells or liver cells, (15) inhibition of eosinophil infiltration or reduction IgE levels in allergic responses or in lung or other tissue, (16) modulation, e.g., a decrease, in serum or blood of leptin levels in, e.g., obese subjects such as humans with a body mass index of about 27, 28, 29, 30, 31, 32, 33, 34 or greater, (17) increased corticotropin releasing hormone synthesis or activity in, e.g., elderly subjects such as humans at least about 60 years of age or at least about 70 years of age, (18) enhancement of memory or reduction of memory loss or disorientation in aging or dementias such as Alzheimer's Disease, (20) enhancement of the synthesis or activity of one or more enzymes responsible for thermogenesis, e.g., liver glycerol-3-phosphate dehydrogenase or malic enzyme, in subjects such as obese or diabetic humans, (21) modulation, e.g., reduction, of the synthesis or biological activity of the CXCR4 receptor or the CXCL12 chemokine in hyperproliferation conditions such as breast cancers or precancers, (22) modulation of the synthesis or biological activity of one or more of holocytochrome c, cytochrome c, second mitochondria-derived activator of caspase, Apaf-1, Bax, procaspase-9, caspase-9,

procaspase-3, caspase-3, caspase-6 and caspase-7, e.g., enhanced translocation of these molecules from mitochondria to cytosol or activation of these molecules in the cytosol in cancer precancer cells, cancer cells or cells that mediate autoimmunity, (23) modulation of the synthesis or biological activity of one or more of tumor necrosis factor-.alpha., interleukin-1.beta. converting enzyme, IL-6, IL-8, caspase-4 and caspase-5, e.g., decreased activation of these molecules in injured cells or cells subject to injury from, e.g., ischemia or infarction (e.g., vascular, cardiac or cerebral), reperfusion of hypoxic cells or tissue or an inflammation condition such as rheumatoid arthritis, ulcerative colitis, viral hepatitis, alcoholic hepatitis, or another inflammation condition disclosed herein, (24) decrease of the synthesis, biological activity or activation of one or more of phospholipase A2, caspase-1, caspase-3 and procaspase-3 in neurodegeneration disorders or dementias such as Alzheimer's disease, Huntington's disease, or another neurological condition disclosed herein. The formula 1 compounds can thus be used where one or more of these conditions or their symptoms is present. Methods to measure the synthesis or biological activity of these molecules has been described, see, e.g., U.S. Pat. Nos. 6,200,969, 6,187,767, 6,174,901, 6,110,691, 6,083,735, 6,024,940, 5,919,465 and 5,891,924.

Summary of Invention Paragraph:
[0912] The formula 1 compounds can facilitating release of myeloperoxidase from granulated neutrophils. The enzyme generates free hydrogen peroxide. Some of the formula 1 compounds, e.g., compounds with a halogen such as bromine or iodine at, e.g., the 16 position, can be metabolized to provide a source of halogen. In cases where the halogen is released, the released halogen can react with hydrogen peroxide (H.sub.2O.sub.2) to generate hypohalogenous acid such as hypobromous acid (HOBr). Exemplary compounds include a halogenated formula 1 compound such as 16-bromoepiandrosterone. Alternatively, a halogen salt, e.g., KBr, NaBr, KI or NaI, can be administered to the subject to provide a source of halogen. The halogen source can be administered to a subject as a component in a formulation that comprises a formula 1 compound or it can be administered separately. Hypohalogenous acid is a potent antimicrobial agent, which may be effective in reducing pathogens in the circulatory system of subjects with a blood cell deficiency who also have a pathogen infection. Hypohalogenous acid that is generated in vivo would provide benefits to such subject as shown by, e.g., a reduced quantitative circulating viral or bacterial culture measurement, without the toxicity that is normally associated with its direct administration to a subject. Biological activities of white blood peroxidase enzymes have been described, see, e.g., M. Saran et al., Free Radical Biol. Med. 1999 26:482-490, W. Wu et al., J. Clin. Invest. 2000 105:1455-1463 and Z. Shen et al., Biochemistry 2000 39:5474-5482.

Summary of Invention Paragraph:
[0917] For example, in lung pneumonitis, administration of a formula 1 compound can lead to detectably increased oxygen saturation in the subject's blood by about 5% or by about 10% or more, e.g., oxygen saturation can rise from about 83% to about 88%, which would typically be detectable by the subject and the health care provider. Such decreased severity of a condition or symptom may be objectively measured in some instances, e.g., by determining the number or activity of circulating platelets or neutrophils or by evaluation of fever, severity or frequency of diarrhea or blood oxygen saturation levels. For other symptoms or conditions, prevention may be subjectively observed by a significant or detectable improvement in a relevant score, e.g., decreased fever or pain or a decreased need for treatment of fever, pain or inflammation.

Summary of Invention Paragraph:
[0925] The formula 1 compounds can be used to prevent, ameliorate, slow the progression and/or reduce the ultimate severity of marrow hypoplasia, hemorrhage, e.g., brainstem hemorrhage, cerebral hemorrhage or gastric hemorrhage or cytopenia, e.g., a blood cell count about 4-25% or more below the low end of a normal range for the subject, e.g., one or more of anemia (e.g., less than about

4.0.times.10.sup.12 red cells/L for adult human females and less than about 4.5.times.10.sup.12 red cells/L in adult human males or a hemoglobin level of less than about 12.0 g/dL in adult human females and less than about 13.5 g/dL in adult human males), late effect leukopenia (e.g., adult human white blood cell counts less than about 3,800, 4,000 or 4,300 mm.sup.-3; adult human basophil counts less than about 10 or 15 mm.sup.-3; adult human neutrophil counts less than about 1,600, 1,800 or 2,000 mm.sup.-3; human eosinophil level less than about 100, 120 or 150 mm.sup.-3; monocyte level less than about 260 or 300 mm.sup.-3) or late effect thrombocytopenia (e.g., human platelet counts less than about 15,000, 18,000 or 20,000 mm.sup.-3).

Summary of Invention Paragraph:
[1134] 12C. The method of embodiment 1C, 4C, 1.degree. C. or 11C wherein the subject's Th1 cells, tumor-infiltrating lymphocytes (TIL cells), NK cells, peripheral blood lymphocytes, phagocytes, monocytes, macrophage, neutrophils, eosinophils, dendritic cells or fibrocytes are activated as measured by, e.g., enhanced .sup.3H-thymidine uptake compared to untreated controls or by an increase in the number of the cell type in circulation or demonstrable movement of the cell type from one tissue or compartment (e.g., skin) to another tissue or compartment (e.g., blood, lymph node, spleen or thymus).

Summary of Invention Paragraph:
[1139] 17C. A product produced by the process of contacting the partially purified or the purified composition of embodiment 16C with one or more sterile containers, one or more syringes, one or more pharmaceutically acceptable excipients (e.g., excipient as defined in draft spec above and including sugars, lactose, sucrose, fillers, lubricants, binders, or any excipient named in any reference cited herein), one or more cells, one or more tissues, plasma or blood.

Summary of Invention Paragraph:
[1147] 25C. A method (e.g., to determine a biological activity of a formula 1 compound or to modulate transcription of a gene in a cell or cell-free transcription system) comprising: (a) contacting the formula 1 compound(s) with a cell or cell population in vitro or in vivo; (b) measuring one or more of (i) a complex between a binding partner and the formula 1 compound, (ii) proliferation of the cell or cell population, (iii) differentiation of the cell or cell population (iv) an activity of a protein kinase C, (v) a level of phosphorylation of a protein kinase C substrate, (vi) transcription of one or more target genes, (vii) enhancement or inhibition of the cellular response to steroids, e.g., glucocorticoids, (viii) inhibition of steroid-induced transcription, e.g., glucocorticoids, sex steroids, (ix) inhibition of retrovirus (e.g., HIV, SIV, FIV or SHIV) LTR-driven transcription, or (x) modulation of the numbers of an immune cell population in circulation in vivo (e.g., circulating peripheral blood lymphocytes in a mammal such as a primate or a human); and (c) optionally comparing the result obtained in step (b) with an appropriate control..

Summary of Invention Paragraph:
[1168] 46C. A method to (a) modulate (detectably increase or decrease) the expression of at least one immune cell antigen by an immune cell in a subject, wherein the immune cell antigen is selected from CD3, CD11c, CD14, CD16, CD19, CD25, CD38, CD56, CD62L, CD69, CD45RA, CD45RO, CD123, HLA-DR, IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, TNF.alpha., IGF.sub.1 and .gamma.IFN, or (b) activate CD8.sup.+ T cells or CD8.sup.- T cells in a subject, wherein the activation comprises at least transiently enhanced expression of CD25 or CD69 by the T cells, or (c) increase the proportion of CD8.sup.+ or CD8.sup.- lymphokine activated killer cells in a subject's CD16.sup.+ cells (e.g., CD8.sup.+, CD16.sup.+, CD38.sup.+ or cells CD8.sup.-, CD16.sup.+, CD38.sup.+), or (d) increase the proportion of (i) CD8.sup.-, CD16.sup.+ natural killer cells, (ii) CD8.sup.+, CD16.sup.+ natural killer cells or (iii) CD8.sup.-, CD16.sup.+ cells that mediate antibody-dependent cell-mediated cytotoxicity, or (iv) CD8.sup.+, CD16.sup.+ cells

that mediate antibody-dependent cell-mediated cytotoxicity, or (e) increase the proportion of dendritic cell precursors in a subject's circulating white blood cells (e.g., Lin.sup.-, HLA-DR.sup.+, CD123.sup.+ or Lin.sup.- HLA-DR.sup.+, CD11c.sup.+ cells) or (f) increase the proportion of CD45RA.sup.+ T cells or CD45.sup.+, R0.sup.+ T cells in a subject's circulating white blood cells, or (g) change (increase or decrease) the proportion or relative numbers of CD62L.sup.+ T cells in a subject's circulating white blood cells, or (h) increase the proportion of CD8.sup.+ or CD4.sup.+ T cells that express CD62L in a subject's circulating CD8.sup.+ or CD4.sup.+ T cells, or (i) decrease the proportion of CD8.sup.+ or CD4.sup.+ T cells that express CD62L in a subject's circulating CD8.sup.+ or CD4.sup.+ T cells, or (j) increase the proportion of HLA-DR.sup.+, CD8.sup.+, CD38.sup.+ cells in a subject's circulating white blood cells, or (k) decrease the level of IL-4 or IL-10 that is expressed by or present in a subject's white blood cells or in a subject's plasma (or that is expressed after the subject's white cells are stimulated in vitro), (l) at least transiently increase the number of dendritic cell precursors or dendritic cells that are present in a subject's white blood cells or in a subject's plasma, or (m) enhance the capacity of an immune cell, e.g., macrophages, CD4.sup.+ T cells, CD8.sup.+ T cells to express IL-2, IL-12 or .gamma.IFN or to activate such cells, the method comprising administering to the subject an effective amount of a formula 1 compound, which is optionally present in a composition comprising a pharmaceutically acceptable excipient.

Summary of Invention Paragraph:
[1189] 66C. The method of embodiment 65C wherein the immune cell antigen is a protein, glycoprotein or cell surface antigen usually or only expressed by lymphoid cells (lymphocytes or white blood cells or their precursors, e.g., T cells, B cells, monocytes, macrophage, LAK cells, NK cells, dendritic cells).

Summary of Invention Paragraph:
[1225] 14D. The method of embodiment 2D further comprising the steps of obtaining blood from the subject before administration of the formula 1 compound and measuring the subject's white or red cell counts and optionally, on one, two, three or more occasions, measuring the subject's circulating white cell counts after administration of the formula 1 compound.

Summary of Invention Paragraph:
[1243] 32D. A method to treat a blood cell deficiency in a subject comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a 25 compound of formula 1.

Summary of Invention Paragraph:
[1277] 63D. Use of a compound of formula 1, e.g., a compound in any compound group or embodiment disclosed herein, to manufacture a medicament for the treatment of a blood cell deficiency, e.g., NP of TP, in a subject, e.g., a mammal or a human.

Detail Description Paragraph:
[1368] The animals were infected with 1-100 TCID.sub.50 units administered intravenously or intrarectally. Viral titers in the first group of animals ranged from 10.sup.6 to 10.sup.8 before dosing began. All animals demonstrated an initial rise in plasma viral SHIV RNA. After a period of 2 to 3 weeks, titers began to decline and 3 of the 4 animals showed a response to therapy with average viral titers of 0.76 log below baseline at weeks 4 to 5 after initiation of treatment. By week 8, titers in all animals had returned to baseline values. Blood glucose levels dropped significantly, alkaline phosphatase levels were elevated and SGOT/GGT values trended towards the high end of normal. No other significant changes were observed in any of the parameters monitored. The CD4 levels in all monkeys remained less than 100 cells/mm.sup.3 at the end of the first protocol.

Detail Description Paragraph:
[1370] The monkeys on this study were salvaged from an infectivity study and the

first cohort of four monkeys on study (Protocol 1) were expected to live only a few weeks past the initiation of these experiments as they were beginning to deteriorate due to disease related causes. One animal died at day 356 from a toxic reaction to the anesthetic used during acquisition of a blood sample for analysis. Survival was greater than 380 days from the time of infection. Treatment by intermittent dosing of the BrEA formulation was used. Three control monkeys were infected with 1-10,000 SHIV.sub.229 TCID.sub.50 units and did not receive treatment. These animals are considered the no treatment arm of a survival study. The mean time to death for pig-tailed macaques infected with SHIV.sub.229 was 193 days. Monkeys receiving therapy remained in good clinical health for over 350 days with CD4 levels less than 20 cells/mm.sup.3 and without opportunistic infections or disease-related symptoms, other than a mild anemia in one animal.

Detail Description Paragraph:
[1373] Animal pharmacological studies. Nonclinical studies were conducted using an oral and a subcutaneous formulation of BrEA. Rats were orally administered .sup.14C BrEA solubilized in different excipients to determine the levels of drug in blood and various tissues. The results of these preliminary pharmacokinetics studies indicated that the absorption of BrEA by oral administration is about 0.1 to 15%, with at least about 80% excreted in the feces.

Detail Description Paragraph:
[1376] A pharmacokinetic analysis of .sup.14C BrEA in plasma was conducted in two female Rhesus Monkeys. Trace labeled compound (16.alpha.-bromo-3-beta-hydroxy-5.alpha.-[4-.sup.14C]-androstan-17-one [50 mCi/mmole]) was used at a dose of 1 mg/kg as a subcutaneous injection in the scapular region using an injection volume of 1 mL/kg. The BrEA was formulated in 25% polyethylene glycol 300,12.5% absolute ethanol, 5% benzyl benzoate, and qs with propylene glycol. 40 .mu.Ci were injected per animal. Blood samples were taken at 0, 0.5, 1, 2, 4, 8, and 24 hours for determination of .sup.14C activity. The radioactivity in the plasma rose to near peak concentration in 8 hours and remained at approximately the same level through the end of the study at 24 hours.

Detail Description Paragraph:
[1377] A pharmacokinetic analysis of .sup.14C BrEA was conducted in New Zealand White rabbits. Twenty .mu.Ci of .sup.14C 16.alpha.-bromo-3-beta-h- ydroxy-5.alpha.-[4-.sup.14C]-androstan-17-one (50 mCi/mmole) plus 1 mg/kg unlabeled BrEA was administered to each of three New Zealand White rabbits as a subcutaneous injection in the scapular region using an injection volume of 1 mL/kg. The drug was formulated in 25% polyethylene glycol 300, 12.5% absolute ethanol, 5% benzyl benzoate, and qs with propylene glycol. Blood samples were taken at 0.5, 1, 2, 4, 8, 12, 24 hours for all three animals, and at 48 hours for two of the animals. Twenty-four and 48 hours after administration, one and two animals respectively, were sacrificed, and the following organs/tissues were collected: brain, heart, kidneys, liver, lungs, skeletal muscle, spleen, and injection site muscle and skin. In addition to the organs and tissues, urine and feces were collected as well as the cage wash. BrEA did not accumulate to a significant degree in any of the organs listed above. Of the organs, the greatest mass of drug was observed in the liver, containing approximately 0.8% and 0.12% of the injected dose at 24 and 48 hours, respectively (average 0.13%).

Detail Description Paragraph:
[1378] The average percentages of the administered dose in whole blood was calculated by multiplying the concentration of drug in whole blood by the assumed volume of blood in the animals, 200 mL. The amount of drug in the blood reaches a maximum at around 8 hours, and a small amount was still evident at 48 hours. The amount of BrEA in whole blood was consistently lower than in plasma, suggesting the drug is not taken up to an appreciable extent by red blood cells.

Detail Description Paragraph:

[1379] In vivo experiments were conducted to determine the bioavailability of BrEA via oral administration using different formulations. BrEA was (1) solubilized in soya oil, vitamin E oil, a mixture of vitamin E and cremophore or (2) BrEA was micronized and combined with or without a surfactant. These formulations are described below. The formulations were administered orally to rats and BrEA levels were determined in the blood, liver, spleen, kidney, and the lymph nodes. In the studies using micronized BrEA, the brain was evaluated for drug uptake. Twenty-four hour urine and feces were collected when BrEA was solubilized in vitamin E and soya oils and vitamin E mixed with Cremophore. The data from these studies indicate that BrEA enters into the lymphatics but is eliminated rapidly from the other tissues. The amount of .sup.14C radioactivity recovered in the feces 24 hours after administration was 78 to 83%. A brief summary of each experiment is provided below and the results are provided in Table 6.

Detail Description Paragraph:
[1380] BrEA (5 mg in 1.0 mL of soya oil or vitamin E oil) supplemented with .sup.14C-labelled BrEA was administered intragastrically to rats. Solubilization of BrEA in the vitamin E or soya oil was facilitated with 50 .mu.L ethanol. Animals (3/time point) were assayed at 1.5, 3, 5.5, and 24 hours after administration and the .sup.14C-radioactivity was measured in the blood, liver, spleen, kidney, lymph nodes and 24 hour feces and urine. The results indicate that, on the basis of .sup.14C-radioactivity, some of the BrEA is taken into the lymphatic system. The uptake is greater with soya oil than vitamin E oil in the blood, liver, and lymph nodes.

Detail Description Paragraph:
[1381] BrEA (5 mg in 1.0 mL of a vitamin E and cremophore) supplemented with .sup.14C-labelled BrEA was administered intragastrically to rats. Solubilization of BrEA in the vitamin E-cremophore mixture was facilitated by the adding 60 .mu.L ethanol. Animals (4/time point) were sacrificed at 2, 3, 5.5, and 24 hours and .sup.14C-radioactivity was measured in the blood, liver, spleen, kidney, lymph nodes and 24 hour feces and urine. The results indicate that a small portion of the drug is taken up by the lymphatic system. Judging from the values in plasma, liver and lymph nodes, it appears that drug uptake is slower compared with soy oil or vitamin E and its presence in the tissues is more persistent.

Detail Description Paragraph:
[1382] Rats, in groups of three males, were orally administered 1.0 mL of 0.9% NaCl containing 10 or 32 mg BrEA micronized with a surfactant, Synperonic PE/F 127 (2.5% wt/wt). Rats were examined at 1.5, 5 and 24 hours after administration. Blood, liver, spleen, kidney, lymph nodes, and brain were assayed for .sup.14C radioactivity. The levels of BrEA in the blood, in comparison to the experiments with BrEA in Vitamin E oil and soya, were higher, 0.3% at 1.5 hours, and increased after 5 hours to 0.8% and 0.9% of the 10 and 32 mg dose, respectively. Additionally, the values in the lymph nodes were similar to those measured at 1.5 hours and the levels were sustained at 5 hours (5.3 and 5.0%) and 24 hours (3.7 and 3.1%) for the 10 and 32 mg dose, respectively (refer to Table 6).

Detail Description Paragraph:
[1383] In a repeated dose experiment, rats were intragastrically administered 1.0 mL 0.9% NaCl containing 2 mg BrEA micronized with Synperonic PE/F 127 (2.5% wt/wt) every 6 to 16 hours. Rats (3/time point) were sacrificed at 40, 72, 84, 90 and 96 hours after the first administration. Blood, liver, spleen, kidney and lymph nodes were assayed for .sup.14C radioactivity. Higher levels in the blood, liver, kidneys and lymph nodes were noted in this experiment over previous studies.

Detail Description Paragraph:
[1384] Rats, in groups of three males, were orally administered 1.0 mL of 0.9% NaCl containing 2, 4 or 10 mg BrEA micronized without a surfactant. Rats were sacrificed at 1.5, 5 and 24 hours after administration and blood, liver, spleen, kidney, lymph

nodes and brain were assayed for .sup.14C radioactivity. The concentration of BrEA micronized without a surfactant in the observed tissues was lower than BrEA plus a surfactant.

Detail Description Paragraph:
[1391] Four-day in vivo protocol for inhibition of Plasmodium berghei. The 4-day suppressive test has been widely used and it can be performed within a 1 week period. The test consists of the inoculation of parasitised erythrocytes on the first day of the experiment (D.sub.0), followed by an injection of the test compound, which is also administered on the 2.sup.nd, 3.sup.rd and 4.sup.th days of the protocol. On the 5.sup.th day, blood films are taken and antimalarial activity is assessed either by calculating parasitemia, or by scoring parasite numbers on a predetermined scale (i.e., 1-5). Peters (Ann. Trop. Med. Parasitol. 64: 25-40, 1970) described a basic procedure using this 4-day test.

Detail Description Paragraph:
[1392] The protocol is summarized as follows. Five female TO mice were used per test group. P. berghei HP15 ANKA parasites were collected by cardiac puncture using a heparinised syringe from a donor mouse having a 30+% parasitaemia. The blood was diluted with diluting agent (50% HIFCS+50% sterile PBS) to a final concentration of 1% parasitaemia or 1.times.10.sup.7 infected erythrocytes per 0.2 mL of the infecting suspension. Each mouse was inoculated intravenously, which produced a more uniform infection rate than intraperitoneal administration of 0.2 mL of the infecting suspension. Test compounds were prepared at doses of 100 mg/kg in (16.7% DMSO +83.3% Celacol). The steroid formulations were administered intraperitoneally 2 hours after parasite inoculation. The compounds were administered once a day starting on D.sub.0, and continued on the following three days. Blood films were made from tail blood on the day after the last dosing of compound and the blood was fixed with 100% methanol and stained with 10% Giemsa. Parasitaemias were scored on a scale of 0-5, where 5 is equal to the control.

Detail Description Paragraph:
[1393] An inoculum of 1% parasitaemia 1.times.10.sup.7 erythrocytes/mL, 0.2 mL per mouse (female strain TO mice), was delivered by intravenous injection. Drug administration commenced 2 hours after inoculation on Day 1 and continued for 3 days. The results are shown below from blood films from all 20 mice on Day 5 when parasitaemias were assessed.

Detail Description Paragraph:
[1394] In a similar protocol, mice are inoculated with a solution containing 1.times.10.sup.7 erythrocytes/mL by I.V. injection. Two hours later give drug is delivered by I.V. injection. BrEA or another formula 1 compound is given (0.2 mL I.V. or S.C.) once a day for 4 days. Tail snips are used to obtain blood after the study. Mice infected with P. berghei were used to obtain infected cells. Parasites are harvested from cardiac mouse blood, and uninfected mice are infected using 0.2 ml of blood with 14% parasitaemia per mouse I.V. Two hours later, the first dose of BrEA (100 mg/kg I.V. or S.C.) is delivered to the infected animals. The BrEA formulation was a sterile solution containing 15 mg/mL of BrEA in 45% hydroxypropyl-.beta.-cyclodextrin and 0.9% saline. At 1, 2, 3 and 4 days after the infection of the animals, BrEA (100 mg/kg I.V. or S.C.) is delivered to the infected animals. No deaths occurred in the group receiving I.V. BrEA at day 30, but all control animals were dead by day 10. All animals treated with BrEA by S.C. delivery were dead by Day 11.

Detail Description Paragraph:
[1395] Rat in vitro and in vivo study. In the in vitro protocol the parasite (Plasmodium falciparum, chloroquine sensitive strain WT and chloroquine resistant strain Dd2) level is adjusted to 1% and the hemocrit is adjusted to 7% with medium. Using a 96 well plate, 50 .mu.L of parasite and 100 .mu.L of drug mixed with media are added to each well and the procedure is done in triplicate. The plate is placed

in a chamber containing a physiological gas mixture and incubated at 37.degree. C. The media/drug mixture is changed at 24, 48 and 72 hours. On day 5 (96 hours) slides of each well are made, stained with Gemsia and 500 red blood cells are counted for each slide. The triplicates are averaged and data are reported in percent inhibition.

Detail Description Paragraph:
[1396] In the in vivo protocol, Lewis rats weighing 80-85 grams were given a standardized IP injection of parasite (Plasmodium berghei). Rats were then intravenously injected 2 hours later with one of the treatments described in the table below, returned to their housing, fed standard lab chow and allowed free access to water. Animals were weighed and treated again 24, 48, and 72 hours after the first treatment and again returned to their housing and they were allowed free access to food and water. The animals were weighed again and then bled using a 26-gauge needle on day 5, 11 and 28 post inoculation. Hemocrits were measured and blood smears are prepared for each rat. The blood smears were then stained using Gemsia and the level of parasitemia (defined as the percent of red cells with parasites) were determined. Animals were again returned to their housing and observed twice daily for evidence of progressive disease, defined as listlessness and or adverse drug reaction, which is defined as a loss of 20% of original body weight, for a total of 28 days. If either progressive disease or drug reaction is noted, the animals are euthanized.

Detail Description Paragraph:
[1400] Venous blood (5 mL) was obtained from two patients before treatment and at 4, 6, 8, 12, 18, 20, 24, 30 and 36 h after treatment or at 4 or 6-hourly intervals after treatment until there was complete clearance of peripheral parasitemia. Blood was collected aseptically and transferred to 10 mL syringes containing 2 mL of acid citrate dextrose (ACD) for in vitro culture. Prior to incubation, the plasma was separated from the red blood cells and the red blood cells were washed twice. Parasites were cultured by modification of standard in vitro culture techniques (W. Trager and J. B. Jensen, Science 193:673-675, 1976; A. M. Oduola et al., J. Protozool. 39: 605-608, 1992). Samples were dispensed into sterile centrifuge cubes within 10 min of collection and spun down. The supernatant plasma was stored while the packed cells were washed twice with culture medium (washing medium, RPMI-1640 medium, containing 25 mM HEPES buffer and 25 mmol/L NaOH). The buffy coat was removed by vacuum aspiration. A 1:10 fold dilution was done for each blood sample with complete washing medium [CMP (washing medium supplemented with 10% human plasma)]. One milliliter each of the sample was transferred into 2 wells of a 24 well micro culture plate. Cultures were incubated at 37 degrees C. in an atmosphere of 5% CO.sub.2, 5% O.sub.2 and 90% N.sub.2 premixed gas. The culture medium was changed daily and thin blood smears were prepared for microscopy at 24 and 48 h after the culture has been set up. The culture samples were diluted with unparasitized washed type A Rh-positive red blood cells if the proportion of parasitized red blood cells was more than 2%.

Detail Description Paragraph:
[1402] During the in vivo study, thin and thick blood films were fixed with dehydrated methanol (100%) and heat, respectively, were stained with 10% Giemsa for 20 min. Parasitemia was quantified in thin films by counting 2000 red blood cells in clear contiguous fields and finding the proportion that was parasitized. In thick films, parasitemia was quantified by counting parasites against leukocytes. A film was declared negative if no parasites were found after examination of 200 microscope fields of a thick smear. During in vitro and ex vivo study, pretreatment thin and thick smears were, graded for ring stages by the method of Jiang as modified by Li et al. (J. B. Jiang et al, Lancet 2(8293): 285-288, 1982; K. Silamut and N. J. White Trans. R. Soc. Trop. Med. Hyg. 87: 436-443, 1993; X. L. Li et al, Chi. J Parasitol. Dis. 12: 296, 1994). Approximately 5000 erythrocytes were counted in clear contiguous fields 24 and 48 h after incubation of blood obtained at each time point and graded for maturity into tiny rings, small rings, large rings,

pigmented trophozoites and schizonts. Functional viability was estimated as the percentage of asexual ring forms capable of maturing to pigmented trophozoites or schizonts after 24-48 h of in vitro culture (W. M. Watkins et al., Trans. R. Soc. Trop. Med. Hyg. 87: 75-78, 1993).

Detail Description Paragraph:
[1404] The patients presented with acute symptomatic severe non-cerebral pure P. falciparum malaria. They had oral fluid intolerance, body temperatures greater than 39.degree. C., greater than 5000 parasites per micro liter of blood, asexual parasitemia and they had a negative urine test for antimalarial drugs. They were administered 25 mL intravenously every four hours with BrEA suspended in sterile 45% .beta.-cyclodextrin in saline at a concentration of 25 mg/mL. This regimen was continued for four days. Parasitemia quantification and clinical examination were done once every 6 hours for the first 72 hours, followed by daily assessment of the parameters up to day 7 (168 hrs) and thereafter on day 14.

Detail Description Paragraph:
[1405] Blood films were Giemsa-stained and parasitemia quantification was done in thick films by counting 2000 parasites against leukocytes, and the thin films by finding the proportion of infected red blood cells. Response to drug treatment was graded according to WHO criteria. Evaluation of therapeutic response was done using the parasitic and fever clearance times. Parasite clearance was expressed as three indices: The time for the parasite count to fall by 50% of the pre-treatment (baseline) value (PC.sub.60); to fall by 90% of the baseline value (PC.sub.90); and to fall below the level of microscopic detection (parasite clearance time) PCT.

Detail Description Paragraph:
[1412] Stimulation of phagocytosis. The capacity of BrEA to influence phagocytosis of Plasmodium parasite-infected RBC is examined using adherent human monocytes. The parasitemia level is about 8-10% and human monocytes are obtained from buffy coats from blood as follows. Peripheral blood mononuclear cells are separated from freshly collected platelet-poor buffy coats discarded from blood samples of heafthy adult donors of both sexes. Separated cells are washed once with luke-warm PBS supplemented with 10 mM glucose (PBS-G) and resuspended at 5.times.10.sup.6 cells/mL in ice-cold RPMI 1640 medium supplemented with 23 mM NaHCO.sub.3 and 25 mM Hepes, pH 7.4 (RMBH). Dynabeads M450 Pan B and Pan T (Dynal) are added to cells in a 4:1 ratio for 20 min at 4.degree. C. B-lymphocytes and T-lymphocytes are removed as specified by the manufacturer. The remaining monocytes are washed 2 times in RMBH, resuspended in AIM V cell culture medium (Gibco) at 1.times.10.sup.6 cell/mL. The monocyte layer is collected, washed with PBS-G at 37.degree. C. and resuspended in AIM V medium at 1.times.10.sup.6 cells/mL. Purified cells are >90% monocytes as assessed by CD14 expression.

Detail Description Paragraph:
[1414] Erythrocyte treatments and parasite cultures are as follows. Fresh blood (Rh+) is used to isolate erythrocytes (RBC). Washed RBC are infected with schizonttrophozoite parasite stages (Palo Alto strain, mycoplasma-free). Stage specific parasites are isolated by the Percoll-mannitol method. Briefly, normal schizont-stage parasitized RBC (SPE) separated on Percoll-mannitol gradient (parasitemia >95% SPE) are mixed with RBC suspended in growth medium (RPMI 1640 medium containing 25 mmol/L Hepes, 20 mmol/L glucose, 2 mmol/L glutamine, 24 mmol/L NaHCO.sub.3, 32 mg/L gentamicin and 10% AB or A human serum, pH 7.30) to start synchronous cultures at selected hematocrit values. The inoculum parasitemia is adjusted to 20% normal SPE for isolation of ring parasitized RBC (RPE) and to 5% normal SPE for isolation of trophozoite-stage parasitized RBC (TPE). At 14-18 hours after inoculum parasites are at ring-stage in the first cycle; at 34-33 hours, parasites are at trophozoite-stage in the first cycle; and at 40-44 hours after inoculum parasites are at schizont-stage in the first cycle. RPE, TPE and SPE are separated on Percoll-mannitol gradients. The parasitemia is usually 8-10% RPE, and >95% TPE. Nonparasitized and parasitized RBC are counted electronically. To assess

total parasitemia and relative contribution of RPE, TPE and SPE, slides are prepared from cultures at indicated times, stained with Diff-Quik.TM. parasite stain and about 400-1000 cells are examined microscopically.

Detail Description Paragraph:
[1417] The formulation containing BrEA is as described herein, e.g., the formulation of example 1 or a formulation that comprises 100 mg/mL BrEA, PEG300-30% v/v, propylene glycol 30% v/v, benzyl benzoate 30% v/v and benzyl alcohol 2% v/v. At day 5-7, if less than about 50% reduction in parasitemia is observed, the patients are given standard care for malaria (mefloquine). During the week of treatment and for 1, 2 3, or more weeks there after, blood samples are taken periodically for evaluation of parasitemia, pharmacokinetics, plasma cytokines (e.g., IL-2, IL-4, IL-10, IGF1, .gamma.IFN, GM-CSF), and intracellular cytokines (e.g., IL-2, IL-4, IL-10, IGF1, .gamma.IFN, GM-CSF). The patients are optionally treated again at about 2 to 12 weeks after the initial dosing, using the same or a similar protocol as that used in the initial dosing protocol.

Detail Description Paragraph:
[1418] An exemplary open-label study of a BrEA formulation administered intramuscularly to semi-immune patients with uncomplicated malaria is conducted. The formulation comprises 100 mg/mL BrEA, PEG300-30% v/v, propylene glycol 30% v/v, benzyl benzoate 30% v/v and benzyl alcohol 2%. Patients will remain at the hospital as in-patients for the first 7 days of the study. Patients will receive one daily intramuscular administration of 50 mg or 100 mg of BrEA for 5 consecutive days. Daily evaluation for the first 7 days, and up to study day 14, may include parasitemia evaluation (twice daily), chemistry, hematology and drug levels (pharmacokinetic evaluation). If, after study day 7, the parasitemia levels decrease from the screening value and the patient is clinically stable, the patient may be followed on a daily basis for parasitemia (twice daily) for up to an additional 7 days as hospital in-patients. If a patient becomes clinically unstable at any time during the study, the patient will be discontinued and may be offered the standard treatment for malaria. Patients deficient in glucose-6-phosphate dehydrogenase enzyme may be excluded, since BrEA inhibits the enzyme. Other considerations that may lead to exclusion of patients from the trial include patients diagnosed with any of the following: severe anemia (hematocrit <21% or hemoglobin <7 g/dL); renal or liver failure by history and/or laboratory results respiratory distress as evidenced by dyspnea or respiratory rate .gtoreq.30 per minute; hypotension (systolic blood pressure <90 mm Hg); tachycardia (heart rate >130 beats/minute); pregnant or breast-feeding women; significant active co-morbid illness (acute medical diagnosis requiring specific therapy; patients with parasitemia >10% on peripheral smear.

Detail Description Paragraph:
[1419] Blood samples may be collected from each patient for future clinical evaluation such as the determination of activation markers or immunological analyses (e.g., assay for intracellular or extracellular interleukins IL-1.beta., IL-2, IL-4, IL-6, IL-10 and IL-12, .gamma.IFN and TNF.alpha.).

Detail Description Paragraph:
[1428] Human HIV clinical protocol. Patients infected with HIV are dosed with an i.m. injection of 25-200 mg of BrEA using a formulation containing 100 mg/mL BrEA, PEG300-30% v/v, propylene glycol 30% v/v, benzyl benzoate 30% v/v and benzyl alcohol 2% v/v. The pateints are dosed once per day for 5 consecutive days followed by a period of about 28 days or longer with no BrEA treatment. The patients were them provided with one more course of 5 consecutive days of dosing with BrEA, followed by a non-dosing period of at least about 28 days. Up to 8 rounds of 5-day treatments, followed by at least 28 days of no dosing were provided. Immunological responses were then assayed using blood or plasma samples from the patients by flow cytometry and other known analytical methods. Immune cell subsets or other measured markers were assayed within 24 hours of obtaining the sample from each patient.

Labeled antibodies, e.g., anti-CD antigen antibodies conjugated with fluorescent dyes (FITC, phycoerythrin, allophycocyanin or PerCP), were prepared and used essentially according to standard protocols using commercially available reagents, see, e.g., PharMingen, 1998 Research Products Catalog, technical protocols at pages 732-774, human cell surface molecules at pages 182-295 and mouse, rat and hamster cell surface molecules at pages 2-173 and cytokine and chemokine reagents at pages 344-489.

Detail Description Paragraph:
[1431] All patients may be monitored for levels of HIV RNA (Chiron Quantiplex.TM. branched chain DNA assay), T-cell subsets [CD4/CD8], proviral HIV DNA (PBMC), interleukins [IL-2, 4, 6, 8, 10, and 12] (serum), .gamma.IFN (serum), insulin-like growth factor [IGF-1] (serum) and tumor necrosis factor [TNF] (serum) throughout the study. PBMC quantitative co-culture (cells) may be conducted on a subset of patient samples. Assays for additional activation markers may be conducted. Analysis of chemistry and hematology panels and urinalysis is planned. Additionally, patients co-infected with hepatitis B and/or C viruses, malaria or tuberculosis may be monitored regularly for viral titers or microbiological cultures. Serial blood and urine samples will be collected from a subset of patients for pharmacokinetic determination after the first dose on Part A and the last dose on Part B.

Detail Description Paragraph:
[1434] Part A will consist of a single intramuscular injection of a BrEA formulation. The day the patient receives the injection will be study day 1. Patients participating in the pharmacokinetic subgroup will have serial blood and urine samples collected, beginning on study day 1. Part B of the study begins on study day 8 (Segment 1) or study day 15 (Segment 2).

Detail Description Paragraph:
[1435] Segment 1 Part B consists of 5 consecutive daily intramuscular injections of the formulation of example 1 at the same dose as received in Part A of the study. The day the patient receives the first dose will be on about study day 8-12. The 5-day treatment course is followed by an approximate 28-day observation period (or approximately 32 days from a first dose on day 8 to the initiation of a second treatment course on day 40). During the observation period, patients will be asked to return to the clinic on a weekly basis for various tests. Patients participating in the pharmacokinetic subgroup will have serial blood and urine samples collected, beginning approximately on study day 12-17.

Detail Description Paragraph:
[1436] Segment 2 Part B consists of 5 consecutive daily intramuscular injections of the formulation of example 2 at the same dose the patient received during Part A of the study. The day the patient receives the first dose will be about at study day 15. The 5-day treatment course is followed by an approximate 45 day observation period (or approximately 49 days from the first dose on study day 15 to the initiation of the next treatment course on study day 64). During the observation period, patients will be asked to return to the clinic on a weekly basis for various tests. Patients participating in the pharmacokinetic subgroup will have serial blood and urine samples collected, beginning approximately on study day 19.

Detail Description Paragraph:
[1440] The results indicated that a single 50 mg or 100 mg dose of BrEA increased the numbers of activated CD8.sup.+ and CD4.sup.+ T cells (e.g., CD8.sup.+, CD69.sup.+, CD25.sup.- cells) that were circulating in the patient's blood. Also, the circulating numbers of dendritic precursor cells, NK cells, LAK cells and cells that mediate ADCC (antibody-dependent cell-mediated cytotoxicity mediated by the CD8.sup.+, CD16.sup.- immune cell subset) functions were increased. Further increases were usually observed on dosing for 5 consecutive days.

Detail Description Paragraph:
[1441] Some of the results are summarized below. Course 1, 2 and 3 refer to each 5 consecutive day treatment regimen of one daily injection with BrEA (50 or 100 mg BrEA per injection). The formulation contained 100 mg/mL BrEA, PEG300-30% v/v, propylene glycol 30% v/v, benzyl benzoate 30% v/v and benzyl alcohol 2% v/v. The data shown below was obtained from patient blood samples at baseline (on the day dosing was initiated) and at various times after the patients received at least one dose of BrEA. The results showed significant increases in immune cell populations and cytokine expression profiles associated with Th1 responses. The patients in this protocol initially had CD4 counts of at least 200 per mm.sup.3 and a serum HIV RNA load of 5,000 to 1.times.10.sup.6 RNA copies/mL. After dosing with one course of BrEA (5 consecutive daily i.m. injections), all patients showed increases in levels of immune cells including activated CD8 T cells (e.g., CD8.sup.+, CD69.sup.+, CD25.sup.-), LAK cells (e.g., CD8.sup.+, CD16.sup.+, CD38.sup.+), NK cells (e.g., CD8.sup.-, CD16.sup.+), ADCC cells (e.g., CD8.sup.-, CD16.sup.+) and dendritic cells (Lin.sup.-, HLA-DR.sup.+, CD11c.sup.+ or Lin.sup.-, HLA-DR.sup.+, CD123.sup.+). Average CD4 IL-10 production dropped from a median of 66% to 4% of the cells, while CD4 IFN.gamma. went from a median of 8% to 63%, leading to a Th2 to a Th1 shift in cytokine production.

Detail Description Paragraph:
[1443] Median activated T cells (CD8.sup.+ CD69.sup.+ CD25.sup.- cells), LAK cells (CD8.sup.+ CD16.sup.+ CD38.sup.+), NK (ADCC responders) cells (CD8.sup.- CD16.sup.+), dendritic cells (Lin.sup.- HLA-DR.sup.+ CD123.sup.+/CD11.sup.+), and cells that mediate Th1 immune responses (IFN.gamma.+white blood cells) compared to baseline cell counts for 3 16.alpha.-bromoepiandrosterone treatments or treatment courses in the HIV-infcted patients gave the following results. The results shown below for the treated patients were obtained about 1 week after the last dose of 16.alpha.-bromoepiandrosterone was administered.

Detail Description Paragraph:
[1448] Whole blood was labeled with a cocktail of four monoclonal antibodies (Becton Dickinson Immunocytometry Systems, BDIS, San Jose, Calif.) per cell subset using allophycocyanin, phycoerytherin, PerCP and FITC conjugates to measure surface phenotypes using a FACSCalibur (BDIS). In addition, four-color immunofluorescent analysis of blood cells was performed on the same schedule. The four color panels consisted of APC, PerCP, FITC, and PE reagents respectively in the following combinations: memory/nave T cells (CD3/CD8/CD45RA/CD62L, CD3/CD4/CD45RA/CD62L), T cell activation (CD3/CD8/CD69/CD25, CD3/CD4/CD69/CD25, CD3/CD8/HLA-DR/CD38, CD3/CD4/HLA-DR, CD38), B cell, LAK and NK (CD19/CD8/CD16/CD38) and dendritic cells (CD11c/HLA-DR/lineage markers CD3, CD16, CD14, CD19, CD56/CD123). Listmode data (25,000 to 50,000 events) were analyzed using FCS Express (De Novo Software, Thornhill, Ontario, Canada). CD3.sup.+ cell subsets were identified by serial gating of (1) nucleated cells, (2) lymphocyte/lymphoblastoid cells, and (3) CD3.sup.+ cells, followed by the gating of the subset of interest. For example, the absolute frequency per .mu.L of any CD4+cell subset (S) was estimated by the equation: S=(proportion of CD4+cells).times.(CD4+cell frequency per .mu.L).

Detail Description Paragraph:
[1449] The CD4.sup.+ cell frequency was determined using the FACSCount test. The absolute frequency of a CD8.sup.+ cell subset was calculated in a similar fashion. The absolute white blood cell (WBC) count was determined using an automated cell counter (Advia 120, Bayer, Tarrytown, N.Y.). The nucleated cell region was defined using a forward versus orthogonal scatter plot. The absolute frequency per .mu.L of CD3.sup.- Natural Killer or Dendritic Cell subsets (F) was estimated by the equation: F=(proportion of nucleated cells).times.(absolute WBC count per .mu.L).

Detail Description Paragraph:
[1482] Blood samples were collected 14, 21 and 34 days after treatment and the sera were analyzed by ELISA to determine the concentration of HBsAg-specific IgG (total

IgG). In addition, samples obtained on day 21 were analyzed to determine the concentration of HBsAg-specific IgG1 and IgG2a subclasses. The results summarized below were average values obtained with blood samples collected 21 days after vaccination of groups of 8 mice. Subcutaneous injection was performed after shaving the hair from the thighs of each mouse. The injected volume was 50 .mu.L for compound (3.0 mg or 0.3 mg) or placebo, and for vaccine preparation. The vehicle control consisted of carboxymethylcellulose (0.5%) in saline (0.9%). Antibody titers were determined by ELISA.

Detail Description Paragraph:
[1489] Modulation of monocyte or macrophage activation or survival. The capacity of the formula 1 compounds to activate monocytes and/or increase monocyte or macrophage activity or survival is determined using methods known in the art. The formula 1 compounds are assayed using, e.g., the assays described below. For these assays, peripheral blood mononuclear cells (PBMC) are purified from a subject, e.g., a human leukopack (American Red Cross, Baltimore, Md.) by centrifugation through a histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation. In each of the assays, the activity of a given formula 1 compound is optionally compared to the response associated with AET or BrEA.

Detail Description Paragraph:
[1490] Modulation of monocyte survival is determined essentially as follows. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated process such as apoptosis. Addition to the culture of activating factors, such as TNF-a improves cell survival. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of about 100 ng/mL of TNF-.alpha. (negative control), and in the presence of varying concentrations of the formula 1 compound. Cells are suspended at a concentration of 2.times.10.sup.6/mL in PBS containing PI at a final 5 concentration of about 5 .mu.g/mL, and then incubated at room temperature for 5 minutes before FACScan analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm. The activity of formula 1 compounds such as AET or BrEA can be used as a comparison standard for other formula 1 compounds.

Detail Description Paragraph:
[1499] Inhibition of a mixed lymphocyte reaction. This assay can be used to evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by formula 1 compounds. Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by the compounds since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells. Compounds that inhibit the MLR are useful in treating, preventing or ameliorating diseases associated with lymphocyte and monocyte activation or proliferation. These include, e.g., inflammatory or autoimmune conditions such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, Crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease and hepatitis.

Detail Description Paragraph:
[1503] Scopolamine induced amnesia is examined essentially as follows. Groups of 13 to 16 C57BL76 mice (about 35 .mu.m) are trained in the Morris water maze procedure to locate a pedestal in less than 15 seconds in three consecutive trials. Immediately upon completion of training the mice in each of three groups are treated with scopolamine (1 30 mg/kg), scopolamine plus a formula 1 compound at one

or more dosages (e.g., about 5-50 mg/kg), and scopolamine plus a placebo. The treatment comprises one, two or three intraperitoneal, subcutaneous, intramuscular or intravenous injections of the formula 1 compound and the vehicle placebo. The injections are given once per day. Six days after treatment the average time (sec) to rescue is timed using the Morris water maze procedure and the results from each group are compared. Results for a formula 1 compound such as 16.alpha.-fluoroandrost-5-ene-17-one or 7-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one are optionally compared to the results that are obtained in these protocols using another control compound, e.g., (S)-(-)--N-propargyl-1-aminoindan or nefiracetam, or another formula 1 compound such as BrEA or AET to determine the relative potency of the formula 1 compounds with respect to each other.

Detail Description Paragraph:
[1505] Briefly, a skin incision is made from the anterior iliac spine to the tip of the scrotum. The testis with cremaster muscle intact is then dissected away from the scrotum. An opening of 1 cm is made on the ventral surface of the cremaster, and the testis and spermatic cord are removed. Under a microscope, the neurovascular pedicle, consisting of the pubic-epigastric arteries, vein, and genitofemoral nerve, is then completely isolated by dissecting to the origin of the vessels from the external iliac artery and vein. The front wall of the cremaster muscle sac is opened and the island cremaster muscle flap is prepared for intravital videomicroscopy. The rat is secured on a tissue bath, and the cremaster muscle flap is spread over the coverglass in the opening at the bottom of the bath and fixed with 5-0 silk sutures. It is then transilluminated from below, using a fiber optic tungsten lamp. The muscle is kept moist and covered with impermeable plastic film. The tissue bath, designed specifically for temperature control, is filled with 0.9% saline and the temperature maintained at between 35-36.degree. C. The microscope is equipped with a color video camera. The video image of the microcirculation is displayed on a 19" monitor, where the final magnification is 1800.times.. Measurement of microvascular activity is recorded after isolation of the muscle to establish the pre-ischemia baseline. After proper positioning of clamps to completely shut down blood flow to the muscle flap, the duration of the ischemic period is six hours. Following removal of clamps to induce reperfusion injury, activity in the microvasculature is measured at e.g., 30, 60 and 90 minutes post-reperfusion. In all experimental subjects, ischemia is followed by reflow and then by an initial period of flow of blood through the microcirculation. This burst of circulatory activity is followed by marked reperfusion injury that induces loss of flow.

Detail Description Paragraph:
[1508] Red blood cell velocities in first order and second order arterioles are measured. Red blood cell velocities are recorded in the main arterioles of the cremaster flap using an optical Doppler velocimeter. Results are obtained for velocity of venous and arterial blood.

Detail Description Paragraph:
[1510] Pulmonary vasoconstriction. The capacity of formula 1 compounds to limit hypoxia induced pulmonary vasoconstriction is demonstrated using an animal model essentially as follows. Isolated perfused ferret lungs are an established animal model to study secondary pulmonary hypertension. In brief, male ferrets are anesthetized with intraperitoneal pentobarbital sodium and the chest is opened. Stainless steel cannulae are secured in the left atrium and pulmonary artery, and the pulmonary artery and the aorta are ligated. The lungs are perfused with a mixture of autologous blood and Krebs-Henseleit buffer in a circulating manner at a constant rate of about 85 mL/min. The perfusion circuit includes a perfusate reservoir, a roller perfusion pump, filter, and a heat exchanger. The perfusion system is made of, e.g., tygon tubing, which is used for connections and for passage through the perfusion pump. The temperature of the perfusate is kept about 37-38.degree. C. and the pH is maintained at 7.35 to 7.40 by adding sodium bicarbonate to the reservoir as needed. The venous reservoir is placed below the

lowermost portion of the lung.

Detail Description Paragraph:
[1516] Blood (0.6-1.0 mL) is obtained from halothane-anesthetized mice by cardiac puncture using a heparinized syringe attached to a 21-gauge needle. Blood is collected in EDTA-containing sample tubes. Mice are euthanized by cervical dislocation after blood collection. White blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts are performed using, e.g., a Hematology System 9000 (Biochem Immunosystems). Wright-stained blood smears from the same samples are made for differential counts of neutrophils and lymphocytes by light microscopy.

Detail Description Paragraph:
[1520] To induce a bacterial infection, all mice are injected sc with K. pneumoniae four days after sham-irradiation or irradiation when circulating leukocytes are depressed. Mice are inoculated sc rather than iv or ip, to establish infection leading to sepsis, but not rapid septic shock. After sc inoculations of K. pneumoniae in the mice, the infection remains largely localized to the injection site. K. pneumoniae are not detectable in blood of inoculated mice until a few hours before death.

Detail Description Paragraph:
[1522] Animals are checked frequently, e.g., once or twice daily, six or seven days per week, to monitor survival and to euthanize mice that are in a moribund state. To verify that mortality in the induced-infection experiments is associated with K. pneumoniae injection, heart blood from recently deceased animals (or moribund animals euthanized by cervical dislocation) is cultured overnight at 35.degree. C. on Columbia sheep blood agar plates (Becton-Dickinson, Sparks, Md.). Colonies are identified as K. pneumoniae by a suitable means, e.g., Biolog analysis.

Detail Description Paragraph:
[1524] To test the ability of formula 1 compounds to ameliorate radiation-induced defects in hemopoiesis, mice are exposed to bilateral whole-body gamma-radiation and receive a dose of 3 Gy (or are sham-irradiated). One hour after irradiation or sham-ir-radiation, mice are injected with 320 mg/kg 3.beta.,17.beta.,7.beta.-dihydroxyandrost-5-e- ne ("AED") or PEG-400 vehicle. Between-group differences in blood cell elements, e.g., neutrophils, GM-CFC and platelets are generally determined. Irradiation results in a decrease in neutrophils at about four days after radiation compared to sham-irradiated animals.

Detail Description Paragraph:
[1527] In patients examined at day 4, 43, 46 and 56 after 1 course of treatment, the level of RNA for several inflammation associated genes was analyzed. The RNA was obtained from circulating white blood cells from the patients and the uninfected volunteers. Peripheral blood was collected into a CPT-Vacutainer (Becton Dickinson) and PBMC isolation was performed according to the manufacturer's protocol. The PBMC were maintained in 1 mL of RPMI 1640 with 10% FCS ar 37.degree. C. for 3 hours and then lysed in lysis buffer (300 .mu.L MagnaPure.TM.). RNA levels from PBMC lysates were measured by preparation of cDNA using commercial AMV reverse transcriptase and PCR kits and protocols (First Strand cDNA Synthesis.TM., Roche Diagnostics; LightCycler FastStart DNA Sybr Green I.TM. Kit, Roche Diagnostics; LightCycler Primer sets, Search-LC, Heidelberg). The results generally showed a detectable decrease compared to baseline levels of about 40-98%, generally about 50-90% for RNA encoding IL-1.beta., TNF.alpha., IL-6, IL-8, IL-10, COX-2 and MCP-1. The level of GM-CSF was increased at 43 days and decreased at all of the other time points. In the HIV-infected patients before treatment with BrEA, compared to healthy uninfected, i.e., not HIV infected, volunteers, there was a statistically significant (Mann-Whitney analysis) increase in RNA encoding IL-1 D, TNF.alpha., MIP-1 cc, IL-6, IL-8, COX-2, M-CSF, GM-CSF, MCP-1 and IFN.gamma.. BrEA treatment for 5 days thus resulted in a decrease in multiple inflammation-associated markers.

Detail Description Paragraph:
[1528] Other details of similar clinical protocols may include at least some of the following procedures. Prior to randomization, patients are optionally screened for acute, active HIV related infections or opportunistic infections. Screening may include vital sign measurements (temperature, heart rate, sitting blood pressure, and oxygen saturation by pulse oximetry), a chest X-ray, a complete physical examination including ophthalmic exam with funduscopy and pelvic examination including a PAP smear, and a complete blood laboratory examination. Additionally, patients may provide sputum samples to identify active TB infection and stool samples for culture and sensitivity for ova and parasites. Patients who require acute treatment, hospitalization, or chemotherapy for active infections are optionally excluded from participation.

Detail Description Paragraph:
[1536] After or during one treatment course of 100 mg of BrEA for 5 consecutive days, the HIV infected patients were examined at days 2-35. Day 1 was the first treatment day. The level of RNA for several inflammation associated genes was analyzed. At day 5, the results showed statistically significant decrease compared to baseline levels of RNA encoding IL-1.beta., TNF.alpha., GM-CSF, MIP-1.alpha. and COX-2, and a statistically significant increase in PPAR.gamma.. The RNA was obtained from circulating white blood cells from the patients and the uninfected volunteers essentially as described in the preceeding example. In the HIV-infected patients before treatment with BrEA, compared to healthy uninfected, i.e., not HIV infected, volunteers, there was a statistically significant (Mann-Whitney analysis) increase in RNA encoding IL-1.beta., TNF.alpha., MIP-1.alpha., IL-6, IL-8, IL-10, COX-2, M-CSF, RANTES, GM-CSF, MCP-1 and IFN.gamma.. At days 2-35 a statistically significant decrease ($p<0.001$ for all markers) in IL-1.beta., IL-6, TNF.alpha., GM-CSF, MIP-1.alpha., MCP-1 and COX-2 transcripts was observed and an increase in PPAR.gamma. ($p=0.034$) was observed. Increases in PPAR.alpha. and t-Bet was also observed in the treated patients.

Detail Description Paragraph:
[1563] Domestic animals, e.g., cows, sheep, swine or goats, are dosed once or twice with about 1 mg/kg, about 5 mg/kg, about 20 mg/kg or with about 50 mg/kg of a formula 1 compound such as BrEA, 3.beta.,17.beta.-dihydroxy- androst-5-ene or 3.beta.,7.beta.,17.beta.-trihydroxyandrost-5-ene by a parenteral route, e.g., by subcutaneous or intramuscular injection, at 1, 2, 3, 4, 5, 6, 7 or 14 days before the animals are to be transported by, e.g., truck or train, from one location to another. The one or two doses are administered on the same day or on different days within the two weeks before the animals are to be shipped and, for animals dosed two times, each dose is the same, e.g., two doses of doses 5 mg/kg or doses 20 mg/kg, or different, e.g., the first dose is doses 20 mg/kg or doses 50 mg/kg and the second dose is doses 1 mg/kg, doses 5 mg/kg or doses 20 mg/kg. Each dose is administered at 1, 2, 3 or more sites. The incidence and severity of stress, shipping fever, weight loss and infection associated with transport is monitored. The capacity of the formula 1 compound to detectably reduce the incidence, severity or rate of progression of an unwanted condition is monitored and is optionally compared to the effects of known agents for the same or similar uses.

Detail Description Paragraph:
[1569] Human HIV-infected patients with impaired or negligible antigen specific immune responses, cell mediated immune responses or delayed-type hypersensitivity immune responses are treated with 16.alpha.-bromoepiandrosterone essentially as descbed herein. White blood cells from the patients are obtained before the compound is administered and at times after an initial treatment and a 2.sup.nd treatment, e.g., dosing of about 50-200 mg at day 1 and at day 19, with assays at about 6-40 days after treatment, e.g., at day 8, 15, 22, 29 and 36. The patients' response to antigens such as HIV p24 and Candida antigen or to phytohemagglutinin is measured. Effects on the patients' antigen specific immune responses or markers

of cell mediated immunity is measured. The capacity of other formula 1 compounds to restore antigen specific or cell mediated immune responses in immune suppressed subjects is characterized in a similar manner.

Detail Description Paragraph:
[1575] Groups of the mice (e.g., about 4-15 animals per group) are injected subcutaneously with one or more dosage of about 1, 2.5, 5, 10 or 20 mg/kg of a formula 1 compound such as 16.alpha.-fluoroandrost-5-ene-17- -one, 7.beta.-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 7.alpha.-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 17.alpha.-hydroxy-16.alpha.-fluoroandrost-5-ene or 17.beta.-hydroxy-16.alpha.-fluoroandrost-5-ene. The compound is administered once daily or once every 2, 3 or 4 days for 1, 2, 3, 4, 5 or 6 weeks beginning either before or at about the time when untreated control animals have developed symptoms or skin lesions characteristic of the disease. The formula 1 compounds can be used with other animal models and markers of psoriasis would be measured essentially as described. See, e.g., B. J. Nickoloff, J. Invest Dermatol. Symp. Proc. 2000 5:67-73, M. P. Schon J. Invest. Dermatol. 1999 112:405-410, B. J. Nickoloff et al., Arch. DermatoL 1999 135:546-552 and M. P. Schon et al., Nature Medicine 1997 3:183-188.

Detail Description Paragraph:
[1583] In any of these treatment protocols, the patient is optionally monitored for the time of appearance of a radiation late effect, its severity. The patient is also optionally treated with other therapeutic agents, e.g., analgesics (e.g., aspirin, ibuprofin, codeine or morphine), corticosteroids (e.g., prednisone or dexamethasome), antibiotics, antifungal agents, growth factors (e.g., erythropoietin, thrombopoietin, .gamma.-interferon or IL-2), blood transfusion or surgery, as the clinical situation dictates.

Detail Description Paragraph:
[1592] Molecules with small and long chain alkyl ethers were also synthesized. The methyl ether (20) of 7-oxo DHEA (2) was prepared in 67% yield by allylic oxidation of 3.beta.-methoxy-DHEA (19) which was obtained by heating 3.beta.-tosyl-DHEA to reflux in anhydrous methanol in 94% yield. Further reduction of the product 20 with sodium borohydride in methanol-dichloromethane (usually 90:10) at 0-5.degree. C. in presence of cerium (III) chloride heptahydrate afforded 3.beta.-methoxyandrost-5-en-7- .beta.,17.beta.-diol (24). The 17-keto group of 3.beta.-methoxy DHEA (19) was protected as ethylene ketal, and subsequent oxidation of the ketal derivative afforded the 7-oxo compound (21). The 7-oxo group of product 21 was further subjected to reduction with sodium borohydride in methanol at room temperature and a mixture of isomeric 7.alpha.- and 7.beta.-hydroxy derivatives (22, 23) were formed, and were separated easily by column chromatography. Reduction of 3.beta.-Methoxy DHEA (19) at position 17, at 0-5.degree. C. afforded 3.beta.-methoxy-17.beta.-hydro- xyandrost-5-ene (27) in 86% yield, which in turn was oxidized at the 7 position using N-hydroxy phthalimide, oxygen and a radical initiator in refluxing acetone to afford product 28 (53% yield).

Detail Description Paragraph:
[1656] Subsequent reduction of the carbonyl group at position 7 of product 21 with sodium borohydride in a mixture of methylene chloride-methanol (1:9) at room temperature afforded 7-hydroxy derivatives. .sup.1H NMR spectrum of the product showed 7.alpha.- and 7.beta.-forms in 2:8 ratio. The diastereomers were separated by column chromatography on silica gel (eluent, ethyl acetate:hexane, 3:7). 3.beta.-Methoxy-17,17-ethylenedioxya- ndrost-5-ene-7.beta.-ol (22) melted at 173-75.degree. C.

Detail Description Paragraph:
3.beta.,17.beta.-di(t-butyldimethylsilyloxy)androst-5-ene-7-on e (33).

Detail Description Paragraph:

3.beta.-(2-tetrahydropyranoxy)androst-5-ene-7,17-dione (37)

Detail Description Paragraph:
[1703] Further elution with the same solvent gave 3.beta.-(2-tetrahydropyr- anoxy) androst-5-ene-7,17-dione (37) as a white solid, which was crystallized from methanol (0.43 g, 68%), m.p. 137-39.degree. C.

Detail Description Paragraph:
[1718] 3.beta.-(1'-ethoxy)ethoxyandrost-5-ene-17-one was oxidized at the allylic 7 position of the steroid using air and N-hydroxy phthalimide as described before to obtain 3.beta.-(1'-ethoxy)ethoxyandrost-5-ene-7,17-di- one (41) in 50% yield.

Detail Description Paragraph:
[1749] 3.beta.-Carbomethoxyandrost-5-ene-7,17-dione (42) (0.5 g, 0.0014 mol) was dissolved in a mixture of dichloromethane (5 mL) and methanol (10 mL). The mixture was stirred at room temperature and finely powdered sodium borohydride (0.16 g, 0.004 mol) was added slowly. After 15 min the reaction mixture was quenched with water and the product was extracted with methylene dichloride. The organic layer was washed, dried and solvent removed. The product was crystallized from acetone-petroleum ether to afford 0.35 g (70%) of 3.beta.-carbomethoxyandrost-5-ene-7,17.be- ta.-diol (49) as a white crystalline solid, m.p. 150-52.degree. C. LC-MS and .sup.1H NMR analysis of the product showed a 8:2 ratio of isomeric 7.beta.-hydroxy and 7.alpha.-hydroxy compound.

Detail Description Table CWU:
28 Induction of the Synthesis of Mitochondrial Glycerophosphate Dehydrogenase and Cytosolic Malic Enzymes in Rat Livers 114 Enzymes Conc. & % of # R b/a b'/a' in diet control Malic * AcO O O 0.040 336 338 9 Glu-ester O O 0.085 166 306 10 Glu-triAc-ester O O 0.107 164 234 12 AcO H/H Glu-ester 0.083 128 229 13 AcO O Glu-ester 0.093 204 363 16 AcO Glu-ester/H AcO 0.071 241 367 17 AcO H/Glu-triAc-ester O 0.046 81 93 18 OTs H/H O 19 MeO H/H O 0.085 241 312 20 MeO O O 0.055 323 424 21 MeO O O-CH.sub.2-CH.sub.2-O 0.042 194 311 22 MeO OH/H O-CH.sub.2-CH.sub.2-O 0.063 395 595 23 MeO H/OH O-CH.sub.2-CH.sub.2-O 0.063 262 324 24 MeO OH/H OH/H 0.055 403 444 25 AcO H/Br O 26 AcO MeO.H** O 0.064 328 459 27 MeO H/H OH/H 28 MeO O OH/H 0.055 322 307 29 MeO O AcO/H 0.06 188 166 30 tert-BuO H/H O 31 tert-BuO O O 0.04 93 116 32 TBDMSO O O 0.036 241 313 33 TBDMSO O TBDMSO/H 0.085 168 380 34 AcO OH/H OH/H 0.046 497 508 35 AcO TBDMSO/H TBDMSO/H 0.082 123 100 36 AcO O TBDMSO/H 0.07 375 382 37 Tetrahydropyran O O 0.064 280 357 38 Dodecanoxy H/H O 39 Dodecanoxy O O 0.068 156 189 40 (Ethoxy)ethyl H/H O 41 (Ethoxy)ethyl O O 0.05 168 275 42 Carbomethoxy O O 0.1 500 786 43 Carboallyloxy O O 0.067 440 384 44 Carboethoxy O O 0.045 265 373 45 Carbo,iso-butyloxy O O 0.048 253 515 46 Carbomethoxy O Carbomethoxy/H 0.051 295 292 47 Carbooctyloxy O O 0.055 256 312 48 Carbo(9-fluorene) O O 0.09 218 310 methoxy 49 Carbomethoxy OH, H** OH/H 0.042 377 359 50 Carboethoxy OH/H OH/H 0.044 277 321 51 Carbooctyloxy OH/H OH/H 0.054 343 260 Each compound was tested in a group of two or three rats. Enzyme activity in the livers of rats fed the stock diet without supplementation is termed 100%. The abbreviation TBDMSO = tert-butyldimethylsilyl oxy. *Mean values from ten experiments (20 rats) in which 7-oxo-DHEA was the comparative standard. **Products tested as diestereomeric mixture (% ratio of .beta. and .alpha., 26, 55:45, 49, 80:20) The following compounds did not induce the liver enzymes: 17-oxoA-3.beta.-glucopyranoside Me ester (8), 3.beta.-acetoxy-17-oxoA-7-g- lucopyranoside Me ester (17), 3.beta.-tert-butoxyandrost-5-ene-1 7-one (30), 3.beta.-acetoxyandrost-5-ene-7.beta.,17.beta.-di-tert-butyldimethyl- silyl ether (35); A = androst-5-ene.

CLAIMS:

1. A method to treat a blood cell deficiency in a subject in need thereof comprising administering to the subject, or delivering to the subject's tissues, an effective amount of a compound of formula 1 115wherein, each R.sup.1, R.sup.2, R.sup.3, R.sup.4, R.sup.5, R.sup.6 and R.sup.10 independently are --H, OH, --

OR.sup.PR, --SR.sup.PR, --N(R.sup.PR).sub.2, --O--Si--(R.sup.13).sub.3, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO.sub.2, --OSO.sub.3H, --OPO.sub.3H, an ester, a thioester, a thionoester, a phosphoester, a phosphothioester, a phosphonoester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an oligonucleotide, a polymer, or, one or more of both R.sup.1, R.sup.2, R.sup.3 or R.sup.4 together comprise an independently selected spiro ring, or one more of R.sup.1, R.sup.2, R.sup.3, R.sup.4, R.sup.5, R.sup.6 and R.sup.10 are .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH.sub.2, or a spiro ring and the hydrogen atom or the second variable group that is bonded to the same carbon atom is absent, or, one or more of two adjacent R.sup.1--R.sup.6 and R.sup.10 comprise an independently selected an acetal, a thioacetal, ketal or thioketal, or all R.sup.3 and R.sup.4 together comprise a structure of formula 2 116R.sup.1 is --C(R.sup.10).sub.2--, --C(R.sup.10).sub.2--C(R.sup.10).sub.2--, --C(R.sup.10).sub.2--C(R.sup.10-).sub.2--C(R.sup.10).sub.2--, --C(R.sup.10).sub.2--O--C(R.sup.10).sub.2--, --C(R.sup.10).sub.2--S--C(R.sup.10).sub.2--, --C(R.sup.10).sub.2--NR.sup.- PR--C(R.sup.10).sub.2--, --O--, --O--C(R.sup.10).sub.2--, --S--, --S--C(R.sup.10).sub.2--, --NR.sup.PR-- or --NR.sup.PR--C(R.sup.10).sub.2- --; R.sup.8 and R.sup.9 independently are --C(R.sup.10).sub.2--, --C(R.sup.10).sub.2--C(R.sup.10).sub.2--, --O--, --O--C(R.sup.10).sub.2--- , --S--, --S--C(R.sup.10).sub.2--, --NR.sup.PR-- or --NR.sup.PR--C(R.sup.10).sub.2--, or one or both of R.sup.8 or R.sup.9 independently are absent, leaving a 5-membered ring; R.sup.13 independently is C.sub.1-6 alkyl; R.sup.PR independently is --H or a protecting group; D is a heterocycle or a 4-, 5-, 6- or 7-membered ring that comprises saturated carbon atoms, wherein 1, 2 or 3 ring carbon atoms of the 4-, 5-, 6- or 7-membered ring are optionally independently substituted with --O--, --S-- or --NR.sup.PR-- or where 1, 2 or 3 hydrogen atoms of the heterocycle or where 1, 2 or 3 hydrogen atoms of the 4-, 5-, 6- or 7-membered ring are substituted with --OH, --OR.sup.PR, --SR.sup.PR, --N(R.sup.PR).sub.2, --O--Si--(R.sup.13).sub.3, --CHO, --CHS, --CH.dbd.NH, --CN, --SCN, --NO.sub.2, --OSO.sub.3H, --OPO.sub.3H, an ester, a thioester, a thionoester, a phosphoester, a phosphothioester, a phosphiniester, a sulfite ester, a sulfate ester, an amide, an amino acid, a peptide, an ether, a thioether, an acyl group, a thioacyl group, a carbonate, a carbamate, a halogen, an optionally substituted alkyl group, an optionally substituted alkenyl group, an optionally substituted alkynyl group, an optionally substituted aryl moiety, an optionally substituted heteroaryl moiety, an optionally substituted heterocycle, an optionally substituted monosaccharide, an optionally substituted oligosaccharide, a nucleoside, a nucleotide, an oligonucleotide or a polymer, or, one more of the ring carbons in D are substituted with .dbd.O, .dbd.S, .dbd.N--OH, .dbd.CH.sub.2, or a spiro ring, or one or more of two adjacent ring carbons in D comprise an independently selected acetal, thioacetal, ketal or thioketal, or D comprises two 5- or 6-membered rings, wherein the rings are fused or are linked by 1 or 2 bonds, or a metabolic precursor or a biologically active metabolite thereof, provided that the compound is not 5-androstene-3.beta.-ol-17-one- , 5-androstene-3.beta.,17.beta.-diol, 5-androstene-3.beta.,7.beta.,17.beta- .-triol or a derivative of any of these three compounds that can convert to these compounds by hydrolysis.

14. The method of claim 7 further comprising the steps of obtaining blood from the subject before administration of the formula 1 compound and measuring the subject's white or red cell counts and optionally, on one, two, three or more occasions, measuring the subject's circulating white cell or red cell counts after administration of the formula 1 compound.

45. The method claim 41 wherein the formula 1 compound is 16.alpha.-bromoepiandrosterone, 16.alpha.-bromoepiandrosterone hemihydrate, 16.alpha.-

hydroxyepiandrosterone, 3.alpha.,16.alpha.-dihydro- xy-5.alpha.-androstane-17-one, 3.alpha.,16.alpha.,17.beta.-trihydroxy-5.al- pha.-androstane, 3.alpha.,16.alpha.,17.alpha.-trihydroxy-5.alpha.-androsta- ne, 3.beta.,17.beta.-dihydroxyandrost-5-ene or 3.beta.,7.beta.,17.beta.-tr- ihydroxyandrost-5-ene, 7-oxodehydroepiandrosterone, 16.alpha.-fluoroandrost-5-ene-17-one, 7.alpha.-hydroxy-16.alpha.-fluoroan- drost-5-ene-17-one, 7.beta.-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 3.alpha.-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 3.beta.-hydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 3.beta.,7.beta.-dihydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 3.alpha.,7.alpha.-dihydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 3.alpha.,7.beta.-dihydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 3.alpha.,7.alpha.-dihydroxy-16.alpha.-fluoroandrost-5-ene-17-one, 7.beta.,17.beta.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 7.alpha.,17.beta.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 7.beta.,17.alpha.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 7.alpha.,17.alpha.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 3.beta.,17.beta.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 3.alpha.,17.beta.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 3.beta.,17.alpha.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 3.alpha.,17.alpha.-dihydroxy-16.alpha.-fluoroandrost-5-ene, 17.alpha.-hydroxy-16.alpha.-fluoroandrost-5-ene, 17.beta.-hydroxy-16.alph- a.-fluoroandrost-5-ene or an ester, ether, sulfate or glucuronide of any of these compounds having a hydroxyl moiety.